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A study of the genetic control of black-red pigment patterns in the fowl

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A STUDY OF THE GENETIC CONTROL OF BLACK-RED PIGMENT PATTERNS IN THE FOWL

by

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INTRODUCTION

The fowl has been used extensively as an experimental animal. Many investigators have delved into the genetics of the fowl, though mainly in search of information that would improve its commercial productivity. Because the embryo of the chicken develops *in ovo*, embryologists have used the fowl extensively for studies of developmental problems. Few workers, however, have combined these two aspects into a developmental genetics approach. Dr. Walter Landauer, one of these workers, pointed out this lack (1952, p. 175):

> The greatest obstacles to rapid progress do not at present lie in the intrinsic difficulties of the problems, although these are great enough, but in failures of organization. Genetic studies on fowl are almost exclusively carried on at the Agricultural Experiment Stations where projects without immediate economic returns find only lukewarm and hesitating support at best. Work on experimental embryology, on the other hand, is rarely undertaken at these institutions and for that reason generally cannot take advantage of genetic breeding techniques. To a large extent, both of these divorced groups of investigators have lacked the co-operation of biochemists. Means must be found in the future to launch integrated projects in which investigators from all three fields join techniques, imagination and ingenuity for an attack on the basic problems of normal developmental genetics.

For this reason, I have attempted to use both approaches--developmental and physiological--in conjunction with the genetic analyses, to discern the mode of gene

action. Since pigment patterns had not been well worked out previously, genetic analyses of the mutants of the Buff Minorca and the E locus were undertaken. Concomitantly, embryonic grafting and other techniques with these types gave physiological data. Part I presents the findings of the Genetic Analyses. Part II presents the Physiological Genetic aspects of black-red pigment differentiation.

PART I. GENETIC ANALYSES

REVIEW OF LITERATURE

Buff

In one of the first recorded genetic experiments involving a buff breed, Hurst (1905) made reciprocal crosses between Black Hamburgs and Buff Cochins. The chicks from both matings were "all blacks marked with brown." In plumage later, the cockerels were quite buffy-brown with spangled breasts. The pullets were black, intermixed with brown and also had spangled breasts. The F_2 population gave a clear segregation of the blacks and non-blacks in the chick, but the adult plumages of both black and non-black types gave an array of various shades of browns, reds, and buffs. Hurst's conclusion was that black was incompletely dominant over buff. His data indicate in reality, however, that more than one locus is involved.

Davenport (1906) crossed a "heterogametous" White Leghorn bantam with a Buff Cochin bantam. His results only showed the heterozygosity of the White Leghorn and exemplified the weak effect of the dominant white factor on red-type pigments. He treated the dominant white and buff as alleles, the white being considered incompletely dominant over buff. He also cited another worker who crossed whites (probably recessive white in this case) and buffs. Ten offspring were

described, revealing nothing of the true genetic components of buff.

In 1909 Davenport again published the results of crosses involving buffs. Crossing a Buff Cochin with a White Silkie, that by previous breeding tests was known to carry the black-breasted red game or wild-type color pattern, he obtained (p. 76):

> ...a washed-out buff color....the Jungle pattern shows itself in the black tail and slightly redder buff of the wing-bar and hackles in the male.

The F_2 chicks were classified as follows: buff, and "buff and black"-34; white-17; game-7. This approximates a 9:4:3 ratio, giving us a general indication of the dominance of the buff factors. The F_2 from the White Leghorn-Buff Cochin cross published in 1906 gave "a very great diversity of offspring," thus shattering the idea of allelism. A cross of Black and Buff Cochins gave results in the F_1 and F_2 similar to those of Hurst, with the exception that a few "whites" were recovered in the F_2. The F_1's backcrossed to the Buff Cochin also produced some "whites."

Davenport suggested that five factors were involved in these various crosses to the buffs. These five factors were:

C-color c-no color
J-Junglefowl pattern j-no Junglefowl pattern
I-Junglefowl pattern with no color

N-supermelanic
X-buff factor (xanthic)
W-graying (white) factor

The gametic genotypes for various breeds were given as follows:

White Silkie	cJnwx
Black Minorca	CJNwx
Black Cochin	CINwx
Buff Cochin	CjnwX

Davenport made some very complex interpretations, for instance, the "I" factor was needed to account for the "whites" obtained in the Black-Buff Cochin F_2.

Proper priority was not given to Davenport's symbols by later workers. Punnett (1923, p. 118) commented:

> The scheme put forward by Davenport (1909) to cover the results he obtained presents many inconsistencies, doubtless inevitable in the state of knowledge at that time....For this reason we shall not attempt to base our account upon his work, although it was the first attempt to express a number of plumage characters in factorial terms.

Knox completely ignored this portion of Davenport's work. Speaking of Davenport and Hurst, he stated (Knox, 1927, p. 117):

> Neither of the above authors attempted to place the inheritance of buff color on a factorial basis.

Consequently, only Davenport's symbol "c" (recessive white) is now in use, but Hutt (1949, p. 546) gave Hadley (1914)

the credit for its introduction.

Goodale (1911) recovered some Rhode Island Red-like birds in an F_2 population from a Brown Leghorn-Buff Plymouth Rock cross. This evidence shows a definite relationship between the buff and red colorations as could have been anticipated in view of the historic origin of American red breeds from Cochin crosses.

Dunn (1922a) compared Columbian and buff color patterns. From the results of his crosses he concluded that the basic difference between these two types was that the Columbian carried sex-linked silver (S) while the buffs did not. The fact that the Columbians show black in wings, tail, and hackle and that the buffs show little or no black was due, he thought, to the selection of modifiers. Later, Dunn (1923) postulated that both breed types carried e^m, "restriction of black." This will be discussed in a later section.

Punnett (1923, p. 138) stated in his review of buff genetics:

> Few experimenters have made use of buff, nor do the recorded results lead us far in the direction of a satisfactory analysis.

In reviewing the work of Hurst and Davenport, he believes that their work is compatible if Davenport's "whites" are considered as equivalent to the "creamy whites" of Hurst, which when grown were buffy colored. He also suggests that

the buff color may be the normal expression of "gold," as he stated (Punnett, 1923, pp. 143-144):

> There are many further points of interest arising in connection with buffs. For example, is buff essentially the same as gold, the differences in tone being due to definite modifying factors? Could we establish a buff race through crossing birds which exhibit gold markings in their pattern, with non-buff breeds?

Only three workers have tried to extract or characterize any specific buff factors. Knox attempted to analyze Buff Orpingtons. His F_1 results were as follows:

> F_1 from Buff Orpington X Black Langshan (Knox, 1927, p. 118):
>
> ...gave only predominantly black individuals in the first generation. The black males carried an intense buff (red?) in the wing bows, back, shoulders and a slight amount in the hackle.
>
> F_1 from Buff Orpington X White Plymouth Rock (Knox, 1927, p. 118):
>
> all of the birds obtained in the F_1 generation were predominantly buff.

He obtained the following F_2 data (Knox, 1927, p. 120):

Parental Breed Crosses	Black	Buff
Buff Orpington X Black Langshan	24	26
Buff Orpington X White Rocks (excluding recessive whites)	12	36

His backcross data were as follows (Knox, 1927, p. 120):

Parents	Black	Buff
F_1(Buff Orpington/Black Langshan) X Buff Orpington	12	82
F_1(Buff Orpington/Black Langshan) X Black Langshan	30	0
F_1(Buff Orpington/White Plymouth Rock) X Buff Orpington	5	141
F_1(Buff Orpington/White Plymouth Rock) X White Plymouth Rock	122	16

These are adult plumage classifications rather than those based on chick down as he notes (Knox, 1927, p. 113):

> ...many of the black chicks develop into birds whose adult plumage would be classified as buffs, while chicks classed as buffs very rarely develop adult plumage which would place them in the black group.

His conclusion from the above data was (Knox, 1927, p. 119):

> It will be noticed that the closeness of fit is extremely poor for the hypothesis that there is only a single factor difference between buff and black. (EE and ee respectively) This is also true when the interaction between Ee and Cc is considered altho to a lesser extent.

He then proposed a "new hypothesis" which suggested (Knox, 1927, pp. 120-121):

> a certain interaction of factors Ee and Cc with two new pairs of genes for buff color. All of the factors concerned are autosomal. The writer has designated these new color factors for buff symbols Bu Bu Bu' Bu'. ...These buff genes are almost wholly recessive to black in an individual having only two doses of buff, and being heterozygous for the extension of black pigment and homozygous for the chromogen factor Cc.

> ...F_2 and back cross data have given verification of this interaction and have afforded the further assumptions; namely, that three or four doses of the buff genes are epistatic to black when acting with CC. From this, it also follows logically that when C is heterozygous two, three or four doses of the buff factors will produce buff color.

This explanation, though unduly complex, fits his data well. We see also that he described only those factors for buff which are epistatic to black (E).

Serebrovsky (1926) crossed black-breasted reds (like the wild-type Gallus gallus) with Buff Orpingtons and Buff Cochins. The F_1 chicks were mostly buff. The females as adults were more golden than the stippled wild type, and the males were red-breasted. He postulated a partly dominant buff factor and called this factor, "Tofa." No report is given of F_2 or backcross matings.

Danforth (1933b) extracted dominant white (I) from Buff Leghorns. This factor, however, does not contribute specifically to the buff coloration.

There have also been reported three factors which, on the basis of logic might be thought to be "buff factors." Serebrovsky (1926) found an autosomal, silver-like phenotype. Its origin is not given. Taylor (1932b) found an autosomal recessive "inhibitor of gold" which also had a silver-like phenotype. Punnett (1948) found a recessive "cream" factor of a similar type.

As we will see, in my data an autosomal recessive factor of this phenotype was not found to contribute to the buff coloration. In summary, we can see, indeed, very inadequate work has been done with buff genetics.

The "E" Locus

The discussion of this locus will consist of four sections. The first section will deal with the major discoveries. The second section will discuss the identification of the various mutants. The third section will point out the common misconceptions and errors involving this locus. The fourth section will review linkage studies involving the E locus.

Major discoveries

Lippincott (1918, p. 112), in discussing "The Case of the Blue Andalusian," postulated a compound locus:

> ...the allelomorphs are two factors, R and E which act on black pigment....E extends any black present to all the feathers of the body.

That is, the blue was considered genotypically Re/rE, black being rE/rE, and blue splashed Re/Re. In 1921, on the basis of crosses among the following breeds, he stated (Lippincott, 1921, p. 324):

> The extension of black pigment to all feathers of the body, resulting, if no pattern factors are present, in self colored individuals, depends upon a dominant factor E. This factor has been found in the Andalusian, [Blue] Orpington, white Plymouth Rock, white Wyandotte and Black Langshan breeds. Some evidence is presented which indicates its presence in white Leghorns.

Dunn (1922b, p. 465) also dealt with black. Basing his results on reciprocal crosses between Black Orpingtons and Light Brahmas (Columbian pattern), he concluded:

> The black fowls have the dominant allelomorph of an autosomal gene (E^m) which determines the extension of black pigment to all parts of the plumage. The recessive allelomorph (e^m) of this gene is present in the Columbian and buff fowls. ...The experimental numbers [from backcrosses] at present are 99 black (E^m): 98 non-black (Columbian or buff e^m).

Later, Lippincott (1923, p. 284), in speaking of Dunn's work, stated:

> Certain matings which the writer [Lippincott] has made for the purpose of studying the hereditary behavior of other characters tend to confirm this [Dunn's] interpretation.

In the same paper he analyzed the difference between E and E^m as follows (Lippincott, 1923, pp. 285-286):

> The writer has reported on a gene for the extension of black pigment in the chicken involved in the hereditary behavior of blue as found in the Andalusian and other breeds. This is also an autosomal gene which has been designated as E. It is a matter of interest to ascertain whether E^m and E are in reality the same gene. The Blue Splashed Andalusian ♂ x Light Brahma ♀ cross, or its reciprocal, should give evidence regarding the situation,

as the Light Brahma does not carry E^m and the Blue Splashed Andalusian does not carry E. Such a cross was made by the writer during the past season with the result that of twenty-three chicks hatched all proved to be self-blue.

Mated with this Blue Splashed Andalusian male at the same time were a Buff Orpington, a Rhode Island Red and a Lakenvelder female. The first two show the Columbian pattern with red replacing the white, while the latter is a white bird with black markings similar to, but not identical with, the Light Brahma, probably a modification of the Columbian pattern.

These three females gave only self-blue chicks in the numbers indicated as follows: Buff Orpington-three; Rhode Island Red-ten; and the Lakenvelder-sixteen. The chicks out of the Buff Orpington and Lakenvelder were blue in the down as well as in the adult plumage, while the chicks out of the Rhode Island Red were classified as black in the down, not becoming noticeably blue until nearly grown.

The most reasonable interpretation of these results seems to be that the Light Brahma, Buff Orpington, Rhode Island Red, and presumably the Lakenvelder, which did not carry E^m, did carry E, while the Blue Splashed Andalusian carried E^m. This indicates that two separate genes for the extension of black pigment were being dealt with. Since none of the F_1 generation showed the Columbian pattern, the Splashed male must have been homozygous for E^m.

Knox (1927, p. 115) failed to grasp the distinction:

> The author believes it is advisable to use the factor EE for the extension gene. This is the same factor that was reported by Lippincott. The writer can see no adequate reason for the use of E^m which was reported later and has the same meaning and action.

Consequently, Lippincott instead of Dunn has been given credit for naming the locus (Hutt, 1949, p. 192):

> Although the recessive gene was first recognized by Dunn and designated as e^m, the dominant allele was earlier considered by Lippincott (1918) responsible for the distribution of blue and black...

Hutt and others have thus simply not realized that Lippincott's compound locus was the source of the confusion, and in ignoring Lippincott's implications they have taken E away from the blue locus and substituted it for E^m. But this newer use seems firmly intrenched in poultry genetics.

Smyth and Bohren (1949, p. 782) identified a third allele at this locus, "ep":

> Crosses between chickens having extended black, New Hampshire (columbian), Dark Cornish and Dark Brown Leghorn color patterns showed that each pattern differed from the others by a single autosomal gene. Crosses in which three or four of these characters were involved simultaneously also segregated in a manner indicating the existence of an allelic series consisting of three and possibly four genes. Black (E) is dominant to columbian (e). The columbian pattern (e) is almost completely dominant to the Cornish but is less completely dominant to the Brown Leghorn pattern. The data are insufficient at this time to determine the relationship of the Cornish and Brown Leghorn patterns.

This was the first indication that multiple alleles were present.

Morejohn (1955), in a very logical and perceptive analysis, introduced evidence for three more E alleles which he symbolized: e^b-brown, e^s-speckled head, and e^y-yellowish-white.

The alleles known by 1955, with their original symbols were as follows: E, e, e^b, e^s, e^y, and ep (wild type).

Mutant identification at the E locus

In conjunction with my own work I have compiled four tables showing the results of various crosses involving the E locus. Included in these tables are data from many other workers. It is obvious, for example, that workers prior to Dunn made crosses involving "E^m." These earlier workers should be given credit for identifying these various E alleles even though their relationships were worked out later. I will mention here only those first identifying these units; Tables 16, 17, 18, and 19, which appear in the RESULTS section under "The E Locus," should be studied for supporting data by other workers.

Hurst's (1905) data on "black" chicks provided the first identification of E (see Table 17). He failed to propose any symbol.

Sturtevant (1912) recovered Columbian types, now called "e," from Brown Leghorn-Columbian backcrosses and F_2. He is the first to provide evidence that such an entity existed.

Morejohn (1955) first identified and symbolized e^b ("brown"). See Tables 16 and 17 under e^p. (This is not the ep of Smyth and Bohren, 1949.)

Morejohn (1955) also identified and symbolized e^s (speckled head). Consult Tables 18 and 19.

Bateson and Punnett (1908) first identified a mutant, "pale brown," probably e^y. See Table 17 under e^y.

The black-breasted red pattern characteristic of the Brown Leghorn, some Game types, and the wild Gallus gallus, presents a special case here since it cannot be considered a mutant. It was symbolized "J" by Davenport (1909), "ep" by Smyth and Bohren (1949) and "e^+" by Kimball (1951).

Allelism tests among these alleles will be discussed in the RESULTS section.

Misconceptions

Much general confusion has arisen in the literature regarding the E locus. Part of the confusion can be attributed to the naivete or inexperience of early workers. As noted in the "Buff" section, Davenport considered buff allelic with dominant white. As another instance, Jull (1932, p. 93) stated that:

> Black is recessive to the white of the White Leghorns. Crosses made by Davenport (1906) and Hadley (1914) between black and recessive white varieties have shown that black is dominant.

If this type of reasoning were carried to its ultimate conclusion, all types would be considered alleles.

A specific error was that of assigning too many breeds the E/E genotype. Lippincott (1923) started the trouble; using his postulated Er/eR blue complex as distinct from Dunn's, E^m, he assigned E/E to a host of breeds, including the Brown Leghorn, Light Brahma, Buff Orpington, and Silver Wyandotte. Danforth (1929b) erroneously stated that the Mille Fleur bantam breed was E^m/E^m and because even by 1948 nobody yet considered the E locus of being multiple-allelic, Hutt (1949) decided to place the Brown Leghorn (wild-type) and partridge varieties in the E/E category.

Another common error was the obverse: that of placing E/E birds in the non-E category. The use of phenotypes rather than breeding tests was the major cause of this error. Danforth (1929b) gave the Golden Sebright the genotype e^m/e^m. Morgan (1919), however, in an F_2 from Golden Sebright X Black-breasted Game, had recovered some black birds, indicating the presence of E in the Sebright. Taylor (1932a) in an F_2 of Rhode Island Red X Silver Spangled Hamburg found 10 extended black to 22 non-extended black adults. His failure to classify the chick down led him to the erroneous conclusion that the Hamburgs were e/e and the Reds E/E. As has been seen in the "Buff" section, Knox (1927) obtained a deficiency of adult black birds in his buff-black crosses. He realized that the buff factors often masked the E expression of the chick in the mature plumage.

Cock and Pease (1951, p. 50) first expressed doubt as to the unifactorial integrity of e:

> But the relation between black-red and Columbian is not simple, as is shown by the crosses made at Cambridge in grading up the autosexing Brussbar (essentially a barred black-red) on Light Sussex. The F_1 birds resemble Columbians, although in some the black pigment is much more extensive; but in backcrosses to the Brussbar the proportion of black-reds (or their silver counterparts) varies widely in different families, and in most is too low to be explained by a single gene.

Kimball (1952a, p. 131) stated in regard to e:

> ...e is a restrictor whose action is clearly not opposed to expression of striping in a number of instances, and might in some cases be interpreted as adjuvant to e^+ in (ee^+) hybrids with high melanin complements. Selection of matings for low melanin complements demonstrates, however, that typical action of e is suppression of striping in (ee^+) heterozygotes.

Practicing the selection he suggested in 1952, Kimball (1956) presented data indicating the dominance and unifactorial nature of e. But the need for selection, itself, admits the multifactorial basis of the phenotype in question. No clear and complete analysis appears to have been made.

Kimball has been the source of much material which confuses the genetics of the E locus. Much valuable information can be gleaned from his data, when presented, but his interpretations leave much to be desired, due to his rather scientifically unconventional approach.

Kimball (1951) suggested a genetic and physiological difference between E black and e^+ black (the nature of the black pigment found in black-red patterned birds). He

clearly stated this supposed difference (Kimball, 1952a, p. 132):

> In view of the fundamental nature of gene action at the E locus, e^+ and its alleles may justifiably be classed as primary pattern genes. A primary pattern gene is defined as a genetic factor determining pterylar or multipterylar distribution of black feather pigment. Alleles at the E locus are distinct in action from secondary pattern genes such as Sg, Pg, and Bg. A secondary pattern gene is defined as a genetic factor determining distribution of black pigment within the individual feather.

Further elucidation of this difference between primary (among feathers) and secondary (within a feather) pattern factors was given by Kimball (1953a, 1954b).

Simply stated, Kimball asserted that factors effecting patterns within a feather cannot be allelic to E.

Morejohn (1955, p. 529) criticized this arbitrary classification by Kimball:

> This classification of gene action on pigment distribution is useful in specific instances but it necessarily becomes useless when attempts are made to apply it to a series of multiple alleles....the fact that the potentialities of alleles of one locus can be extremely variable (affecting the same character in different ways) has been shown by studies on other forms....Alleles e^b and e^s, although producing nearly identical adult phenotypes, are clearly distinct in their action on down plumules. These can be classified as secondary pattern genes.

Kimball's classification causes errors in assigning genotypes. The e^+e^+sg sg yields black birds and can be misclassified with E birds because they lack the wild type

stippling factor, (Sg), according to Kimball (1952a). Buttercup (Kimball, 1953b), Dark Cornish, Wyandotte (Kimball, 1955) and Wheaten (Kimball, 1960) phenotypes he considered automatically of the e^+e^+ genotype. Allelism tests which should have been made were neglected because allelism was thought impossible.

Kimball's second hypothesis suggests that the E locus is a complex (Kimball, 1954a), its components being B-black and R-red [B-R]. Various combinations of B, b, R, and r were postulated to produce the expressions of E, e^+, and e.

To the already existing series he added E^R (Kimball, 1954a) for the Birchen phenotype, but performed no standard allelism tests. Further explanation of his "gene-cluster" theory was stated in 1956. He assumed without data that Sebrights also carry the E^R allele (Kimball, 1955).

Linkage of the E locus

Neither Hutt (1949) nor Warren (1949) recorded any linkage tests involving the E locus. Morejohn (1955) demonstrated that there was no linkage between the E locus and pea comb (P).

MATERIALS AND METHODS

I used the wild type (Red Junglefowl) as the standard of reference in all of my analyses.

The genetic analysis of a particular phenotype should be able to: (1) show dominance relationships, (2) determine the number of factors involved, (3) indicate the nature of gene action, and (4) clearly distinguish interactions. Such an analysis requires a genetic standard of reference. The ideal standard is wild type. Jaap and Hollander (1954, p. 99) proposed:

> The analytical approach used in the genetics of Drosophila, whereby the wild type serves as a standard of reference and all genotypes are expressed in terms of deviation from it is recommended for poultry research and teaching.

They indicate that the Red Junglefowl (Gallus gallus) is the normal or wild type of the domestic fowl suitable for this purpose. Deviations from this type are referred to as abnormals or mutants.

Any mutant is considered dominant, semi-dominant, or recessive depending on its behavior in crosses with the standard. If the F_1 looks normal, the mutant is recessive; if the F_1 looks abnormal, the mutant is dominant or semi-dominant. The number of factors involved in a particular phenotype can be determined from ratios, or even simply by

noting the fraction of wild types recovered in the F_2, and backcross or testcross. The nature of gene action can be inferred by examining the deviant phenotypic effect (grossly or otherwise) of the mutant when it is expressing itself alone on the wild-type background, as well as in mutant combinations. Interactions can be understood, by placing the two or more mutants in question on the wild-type background. These interactions can often be predicted when the nature of the individual gene action has been worked out.

Analyses of the Buff Minorca and E locus were undertaken. Various other types (e.g., Rhode Island Red) were investigated in connection with their relationship either to the buff or to the E locus. The procedure for isolating the "dominant" mutants from buff is outlined in Figure 1. Analysis of the multiple-allelic E locus consisted mainly of testing factors for allelism. Identification tests were also made (see Tables 16 and 17). Since the only recessive mutant recognized in the buff was an allele at the E locus, its characterization is included with our E locus analysis.

Three possible combinations of factors are possible. The first is a combination of two recessive mutants. If they are at different loci a wild-type phenotype is the result. If they are alleles, one of the two phenotypes or an intermediate type will express itself. This combining of two recessives is in itself an allelism test; however, for

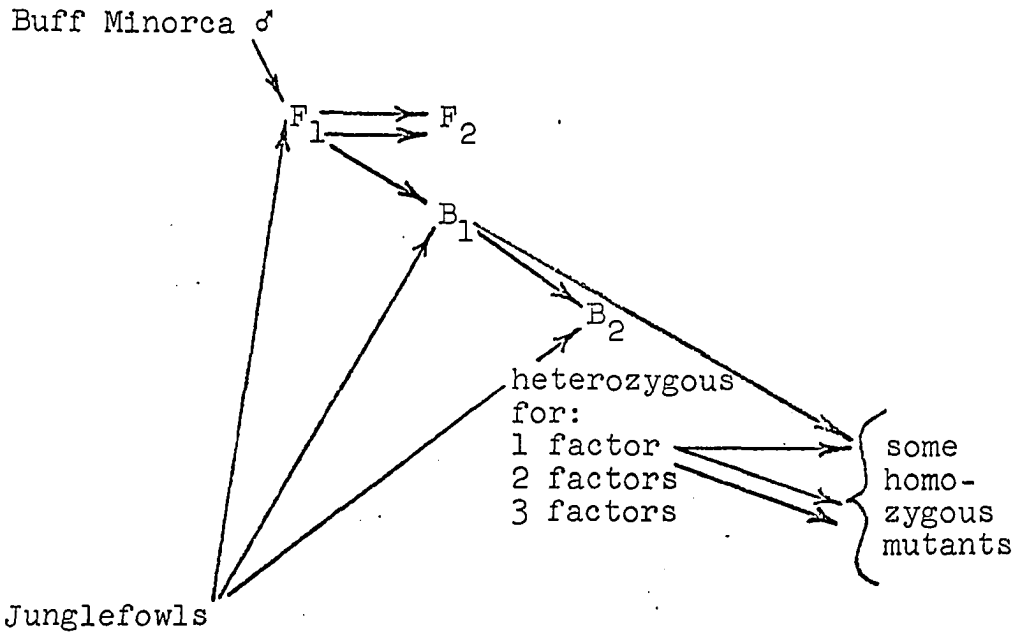


Figure 1. Method used for the analysis of the Buff Minorca

Pedigree # sex Wing band #

I.1-feet & legs(color,mutants,etc.)
2-comb(+,pea,rose,etc.)
3-down:a-head,b-back(nature of striping,etc.)
4-ground color
5-other mutants
6-special remarks
hatching date

II.(any changes in the above;plumage color in category 3.)
culling date

Figure 2. Individual record card

complete testing a backcross of the heterozygote to the most recessive and/or an F_2 of the heterozygotes should be made. A recovery of wild-type progeny from any of these types of matings classically shows they are not alleles.

The second combination in which suspected allelism is to be tested is that of a dominant mutant with a recessive mutant. The initial combination is not an allelism test as the dominant phenotype will be expressed. A backcross to the recessive or an F_2 with the recovery of wild-type progeny disproves allelism.

The third combination is that of two dominant mutations. An allelism test would consist of an F_2 and/or of a backcross to wild type. Recovery of wild type would again demonstrate that they are not alleles. If available or known, a cross of the heterozygote to a recessive known to be an allele of one of the two dominants would also be an allelism test.

On the basis of the above reasoning, various combinations of mutants were tested for allelism at the E locus. The matings used are shown in Tables 18 and 19.

Ratios were tested for closeness of fit, when necessary, with the standard chi-square test. F_2 data with possible linkage were tested with chi-square and if linkage was suspected an estimate of the linkage distance was determined using Immer's (1930) tables.

Although some natural matings were made in small floor

pens, most of the birds were placed in individual cages and mated using the artificial insemination technique of Burrows and Quinn (1939). Because the Junglefowl and Junglefowl crosses were smaller than the "average" domestic chicken, only one operator was required to carry out the insemination procedure. The hens were inseminated once a week. Birds were mated as soon as feasible after they were sexually mature. Eggs were set every two weeks throughout the year. At hatching the chicks were carefully described, each on a 3" x 5" card (see Figure 2). Pedigree numbers were determined as follows: each mating was assigned a number, each successive hatch was assigned a letter and each chick in that hatch was assigned a number. For example, bird 101C5 was from mating 101, the third hatch, the fifth chick described of the hatch.

Chicks that were to be kept were wing-banded. Samples of each type of chick were killed and the skin preserved flat with Borax for a permanent record. The unneeded chicks were killed and opened to determine their sex. At four weeks of age the living birds' upper beaks were clipped by electro-cautery to prevent feather picking and cannibalism. At approximately eight weeks of age the birds were again described using the same card and the same scheme as that used at hatching with plumage color replacing the third category--down pattern. Most of the birds were then

disposed of except those being saved for further breeding tests. Samples of feathers from various body regions of each characteristic type were kept for a permanent record.

The flock was fed and watered daily. Chicks received a commercial starter ration. Mature birds were fed a commercial laying ration.

Diseases did not seriously affect this study, since most of the birds were raised and kept on wire floors and weekly fed a supplement of powdered "calf milk replacer." A few isolated instances of fatal coccidiosis and avian leukosis were the chief disease losses.

The following genetic stocks were used:

Red Junglefowl--from 1 ♂ and 2 ♀♀ the second generation from a cross of 2 strains, one of which had been obtained from Dr. Walter Landauer of the University of Connecticut, the other from the Lincoln Park Zoo in Chicago, Illinois. The F_2 birds were individually progeny tested for homozygosity of color-pattern factors. The chicks are well striped (see the "+" column in Figure 29 which appears in the RESULTS section under "The E Locus"). The adult ♂ is pictured in Figure 3. The adult ♀ is pictured in Figure 4.

Black Castilian--several ♀♀ from the Poultry Department, Iowa State University. The chicks are black with white ventrally and wing tips (see "E" column in Figure 29). The adults are black.

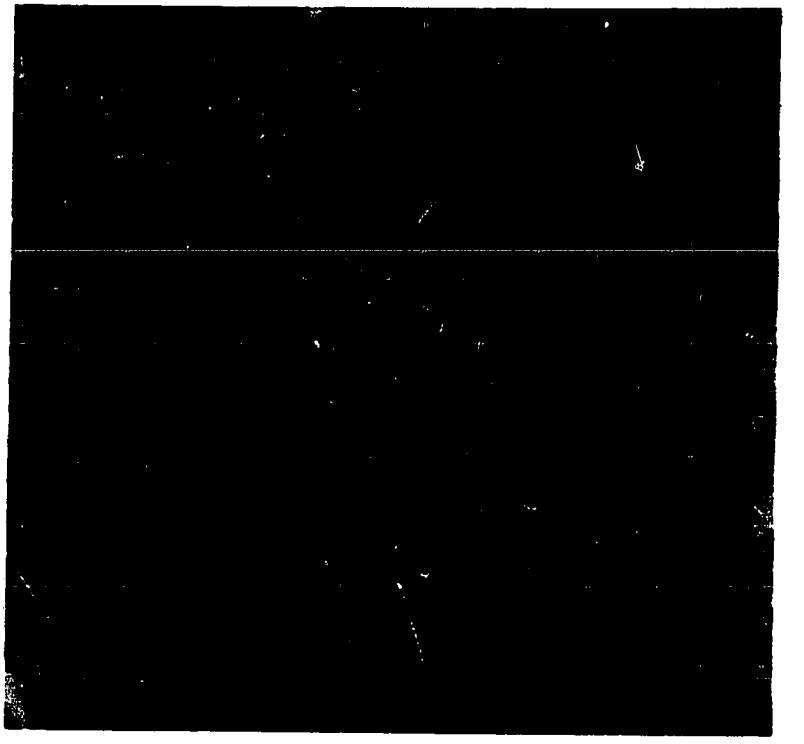


Figure 3. Junglefowl ♂

Figure 4. Junglefowl ♀

Brown Leghorn (Dark Rose comb)--1 ♀ from McMurray Hatchery, Webster City, Iowa. Chicks may be striped or brown. The adults are near wild type but yellow shanked and with a deeper red in the red areas and more black shafting in the hackle.

Brown Leghorn (Light)--1 ♀ from McMurray Hatchery, Webster City, Iowa. This type is essentially wild type but with yellow feet.

Buff Minorca--1 ♂ from McMurray Hatchery, Webster City, Iowa. Chicks are buffy-yellow with a brownish beak. The adults are buffy-yellow throughout. The shanks and feet are white.

Buttercup--1 ♂ and 1 ♀ from McMurray Hatchery, Webster City, Iowa. Chicks are spotty-headed on a buffy ground color with a faded, shortened dorsal back stripe. The adult ♂ is pale red. The adult ♀ is rather buffy with black ovals, stretching from the shaft to the edge of the feather and spaced at regular intervals the length of the body feathers (basically a barring pattern).

Partridge Rock--1 ♀ from McMurray Hatchery, Webster City, Iowa. Chicks are brown. Juveniles are barred black and red. The adult ♂ is a dark standard. The adult ♀ exhibits concentric rings of black and red on her body feathers.

Rhode Island Red--several ♀♀ of Parmenter strain

from Poultry Department, Iowa State University. Chicks are pale red with a brownish beak. Adult ♂♂ and ♀♀ are mostly red with black in wings and tail. These were production-bred rather than show stock.

Salmon Faverolle--1 ♂ recessive white segregate from a breeder. The typical chicks of this breed are yellow with a dark speck on the back of the head and a spot on the back. The adult ♂ is standard but with white ("silver") replacing part of the red areas. The adult ♀ is light silvered red with black in wings and tail.

Silver Pencilled Wyandotte bantam--1 ♀ from McMurray Hatchery, Webster City, Iowa. The description is the same as that of the Partridge Rock but with the red areas replaced by white ("silver").

Silver Spangled Hamburg bantam--1 ♀ from McMurray Hatchery, Webster City, Iowa. Chicks are silvered pale blackish. Adults are white ("silver") with black-tipped ("spangled") feathers.

Speckled Sussex--1 ♀ from McMurray Hatchery, Webster City, Iowa. Chicks are pale red with white ventrally and wing tips and a brownish beak. The adults are mostly red with white-tipped body feathers.

Wheaten Game bantams--several ♀♀ from a breeder. Chicks are yellow with a head speck and a back spot. The adult ♂ is wild type. The adult ♀ is pale red with black

in wings and tail.

$\underline{E/e^{y}}$--2 black ♀♀ (2133, 2182) from a black F_2 ♀ (Silver Spangled Hamburg ♂ X pink-eyed pale red ♀) crossed with a wheaten ♂.

These and several other stocks from Dr. Hollander were used (see Figure 5 for their identification and origin).

For a more detailed description of the breeds mentioned consult the American Standard of Perfection (1953).

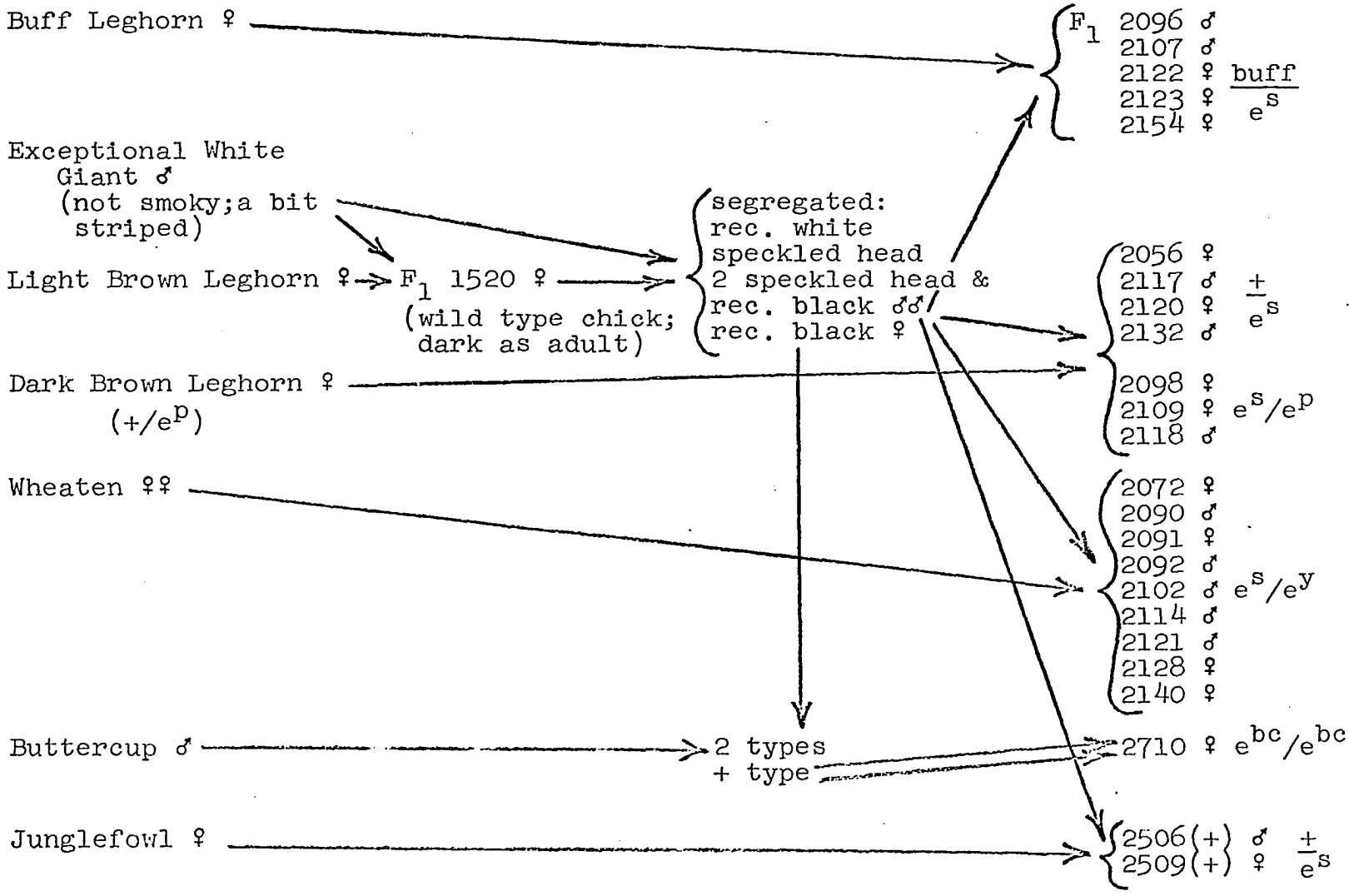


Figure 5. Pedigree chart showing the origins of some of the stock used

RESULTS

Buff Minorca Analysis

Introduction

Four previously unrecognized dominant or semi-dominant factors have been isolated from the Buff Minorca, namely: ginger, mahogany, dilute, and champagne blonde. An autosomal recessive was also found, which will be discussed in a later section. The F_1 type, these mutants and some of their interactions will be presented.

The F_1 generation

An F_1 chick skin is pictured in Figure 6. Notice that the striping, although very narrow, is quite evident. The dominance of the "buff factors" is indicated by the buffy color of the down. The F_1 chicks also displayed a dark brown upper beak. The F_1 adult male shown in Figure 7 also shows the dominance of the "buff factors." Notice that he is predominantly buff but red in the wing bows and black in the flights and tail. The F_1 female (Figure 8) is also mostly buff, but exhibits a good deal of black stippling or patterning in back, wings, and tail. Interestingly, the F_1 adults have light blue shanks and feet. This indicates that

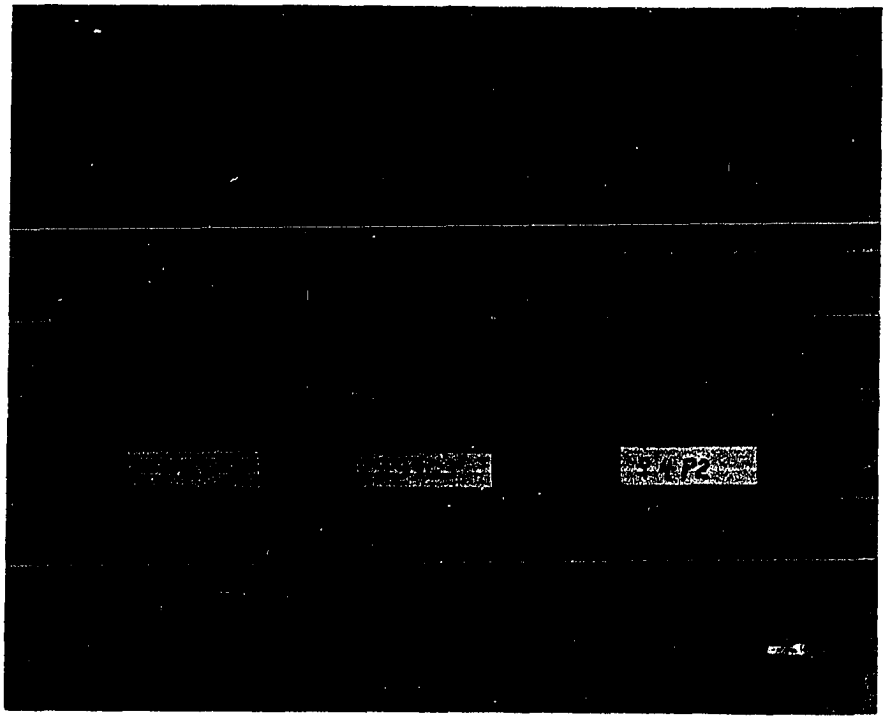


Figure 6. +, F_1(R1R/+), and F_1(Buff Minorca/+) chick skins

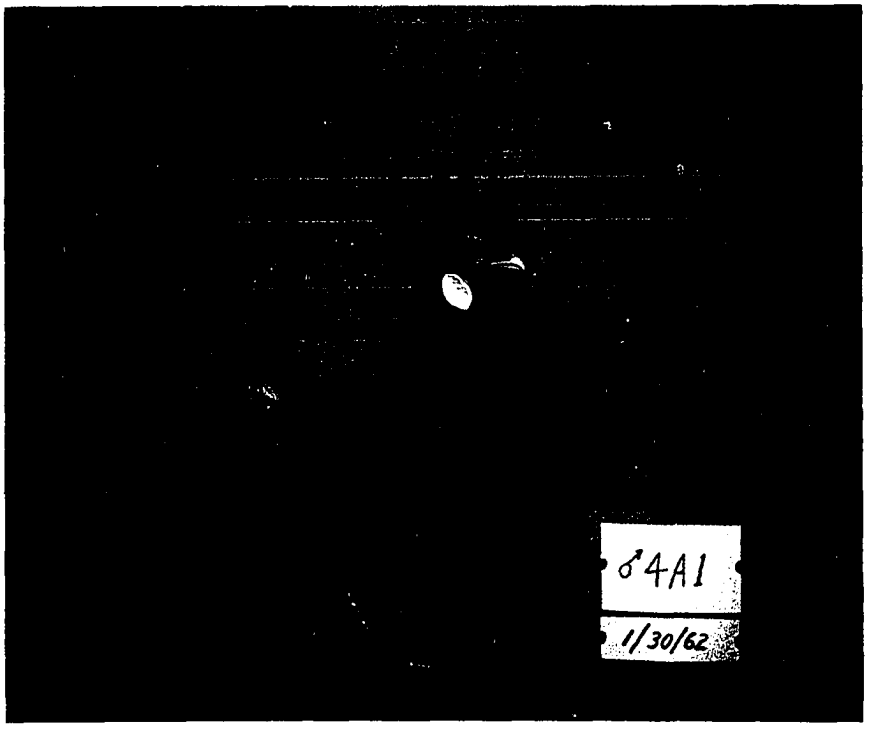


Figure 7. F_1 ♂ (Buff Minorca/+)

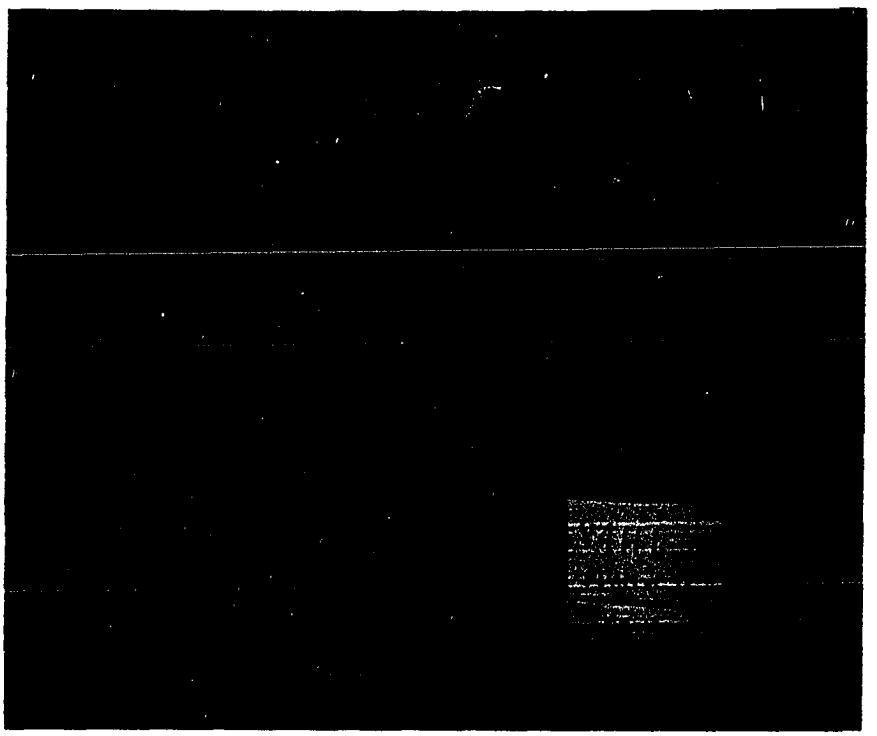


Figure 8. F_1 ♀ (Buff Minorca/+)

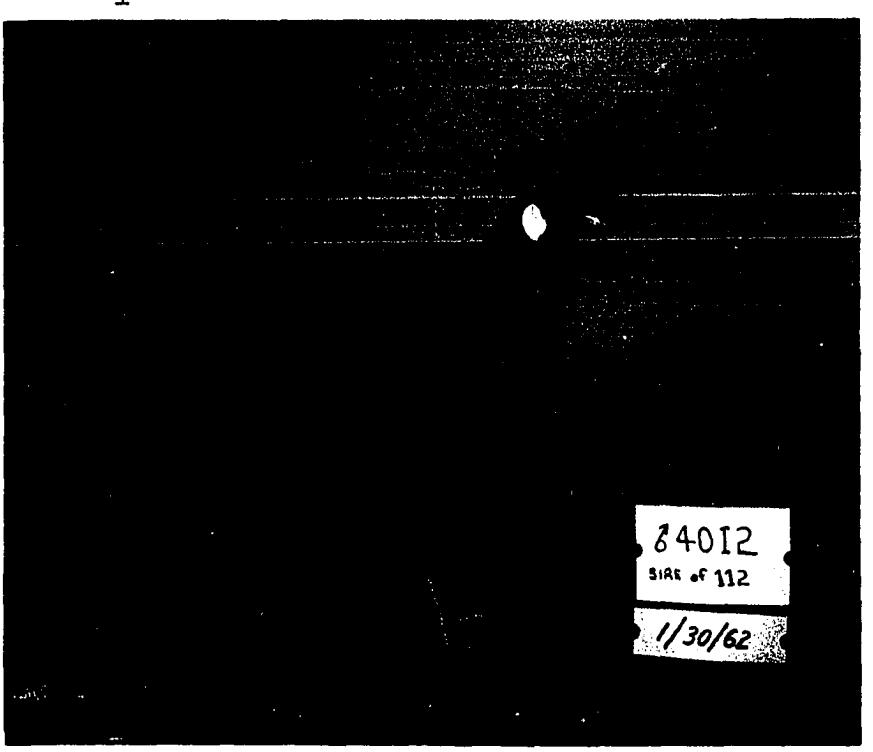


Figure 9. Gr/+ ♂

the sex-linked inhibitor of dermal pigmentation, Id, is not present in the Buff Minorca breed. In all, 63 chicks were examined and 47 of these were later examined in the adult plumage. They were quite uniform.

The first backcross generation

Adult plumages of 277 birds from this generation were examined. The females were F_1 colored, red, red with black stippling, pale, and some, of course, wild-type. The males were buff, red, red-and-black breasted, buff-and-black breasted, and some wild type. The variation was quite extensive.

Ginger

The typical ginger heterozygote chick has a dark brown upper beak. The down striping pattern is essentially wild type, but the non-black portions are tawny, which is especially evident ventrally. The typical adult male (Figure 9) has more gingery than wild type red. His breast feathers are reddened in an hour-glass pattern. The typical female (Figure 10) has coarse very dark stippling in back, wings, and tail. The fluff of the female body feathers is black to dark gray. Birds with this phenotype segregated clearly in backcrosses to wild type (see Tables 1 and 2).

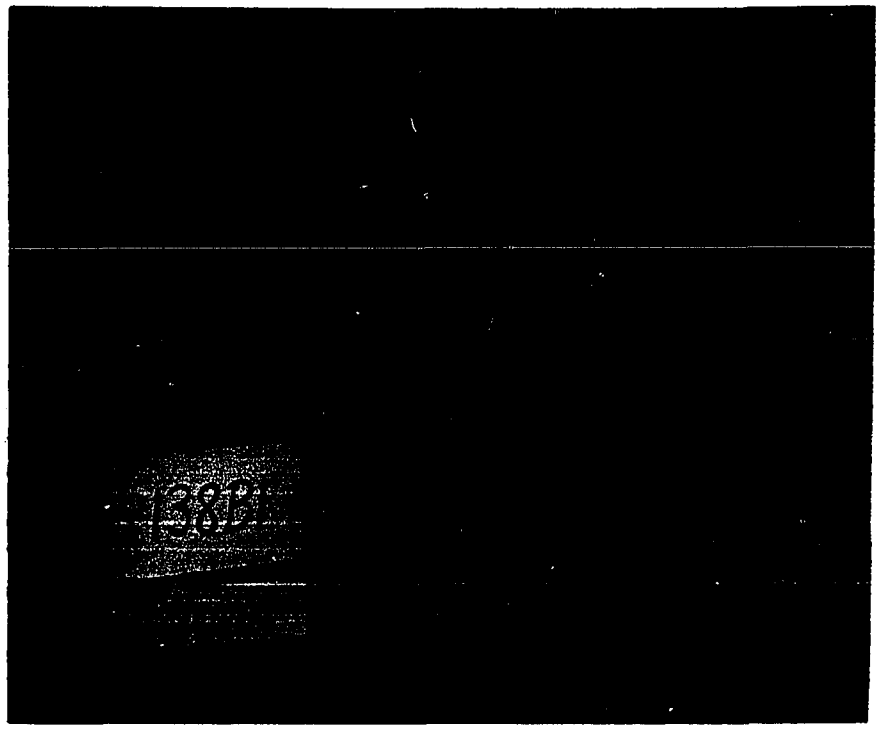


Figure 10. Gr/+ ♀

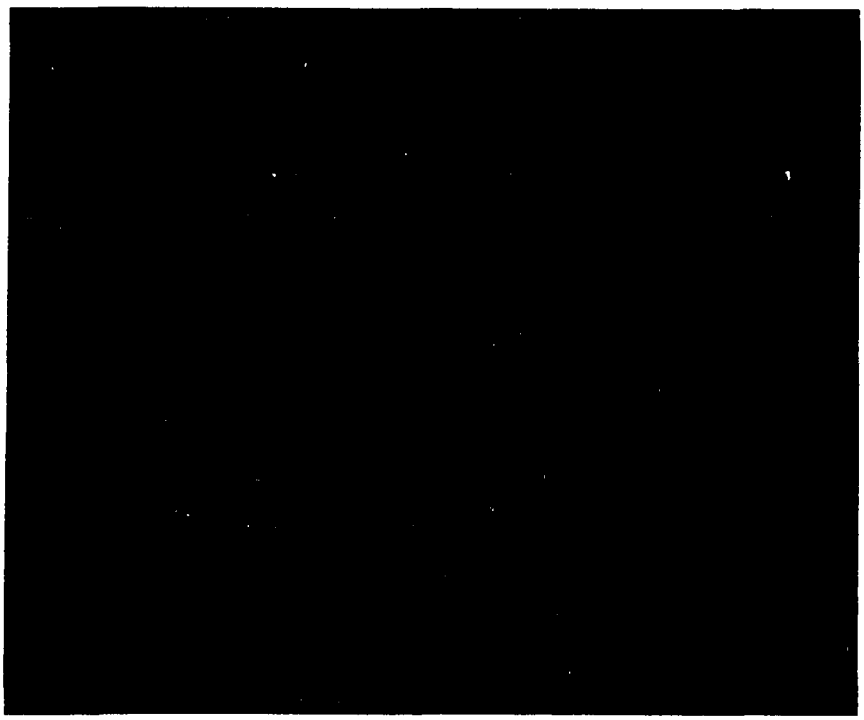


Figure 11. Gr/Gr, +, Di/Di chick skins

Table 1. Second backcross matings for segregation of ginger (baby chick)

Mating #	Mutant parent	Progeny		
		Gr/+	+/+	Total
97	18I2♂	11	14	25
112	40I2♂	17	11	28
123	40H2♂	13	22	35
138	54F2♂	30	26	56
Total		71	73	144
Expected		72	72	

Pooled chi-square = .03
Tabular chi-square (df = 1; .05 level) = 3.84

Table 2. Second backcross matings for segregation of ginger (plumage)[a]

Mating #	Mutant parent	Progeny		
		Gr/+	+/+	Total
97	18I2♂	6	7	13
112	40I2♂	14	10	24
123	40H2♂	15	17	32
138	54F2♂	27	23	50
Total		62	57	119
Expected		59.5	59.5	

Pooled chi-square = .22
Tabular chi-square (df = 1; .05 level) = 3.84

[a]Totals different than those of Table 1 due to mortality losses.

Matings of ginger heterozygotes produced three classes of progeny approximating a ratio of 1 ginger homozygote : 2 ginger heterozygotes : 1 wild type (see Table 3).

The typical ginger homozygote chick (Figure 11) has a very dark brown upper beak. The down pattern which is essentially wild type has a brownish-buffy overcolor with a tawny belly. The typical adult male (Figure 12) is mostly light red with black in the flights and tail. The typical female (Figure 13) is also mostly light red with black in the flights and tail and with some black stippling over the back. The fluff of her body feathers is black to dark gray.

Table 3. Matings of ginger heterozygotes for homozygotes

Mating #	Sire	Dam	Progeny			
			Gr/Gr	Gr/+	+/+*	Total
192	40H2	102D5	14	9	15	38
202	54F2	138B1	14	28	15	57
214	54F2	112C4	6	7	2	15
232	54F2	138G4	8	14	9	31
Total			42	58	41	141
Expected			35.25	70.50	35.25	

Pooled chi-square = 4.46
Tabular chi-square (df = 2; .05 level) = 5.99

*Both sexes represented.

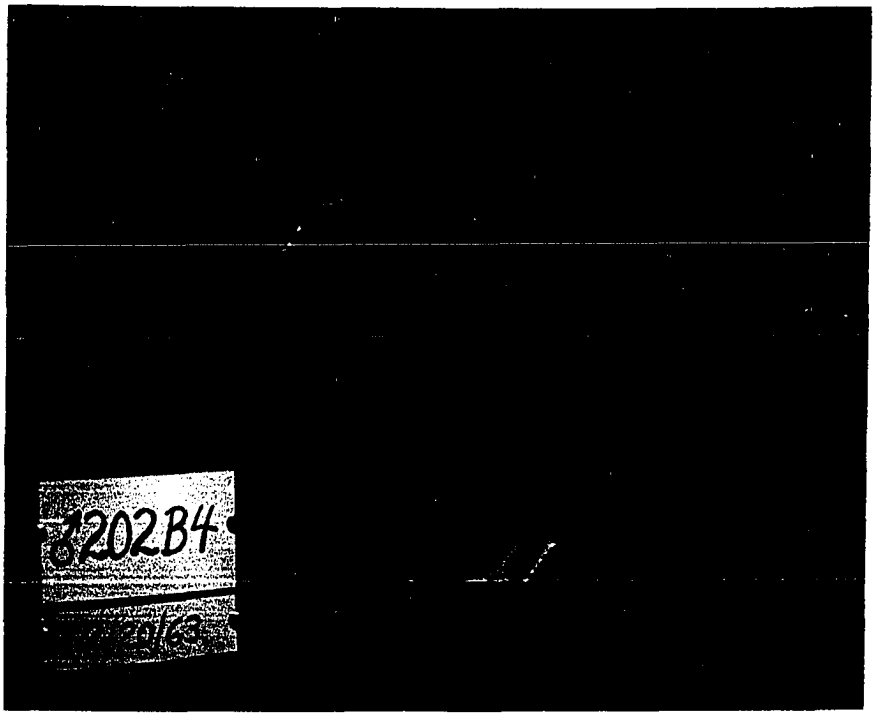


Figure 12. Gr/Gr ♂

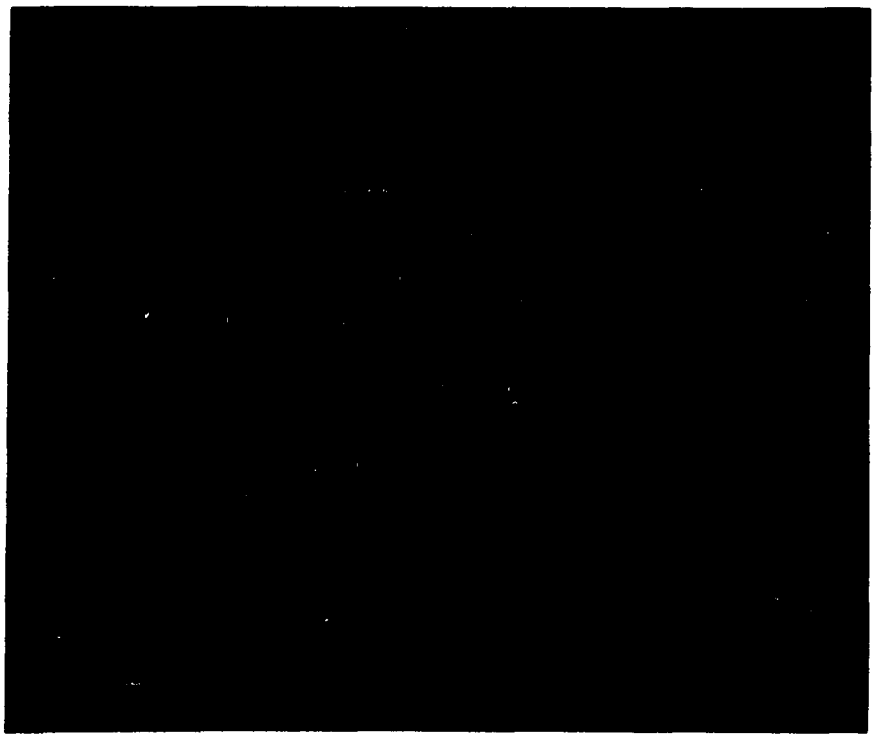


Figure 13. Gr/Gr ♀

Tegetmeier (1873, p. 262) described a color variety of Game fowls called ginger-red. His description resembles the above-mentioned homozygous mutant. The name is still current among cock fighters, and an old-timer of Ames used the term "ginger" when shown this mutant. Therefore this mutant is named ginger and given the symbol Gr.

Mahogany

The mahogany heterozygote chick is indistinguishable from wild type. The typical adult male (Figure 14) has "spangled" breast feathers (black tip with red base). The size of the spangle varies between individual birds. It may just appear at the very tips of the feathers, or it may include almost the whole feather leaving just a red shaft. The typical adult female (Figure 15) exhibits a slight reddening of all body feathers, particularly evident in the breast and wing bows. Birds with this phenotype segregated clearly in backcrosses to wild type (see Table 4).

Matings of mahogany heterozygotes produced 3 classes of progeny approximating a ratio of 1 mahogany homozygote : 2 mahogany heterozygotes : 1 wild type (see Table 5).

The typical mahogany homozygote chick is nearly wild type. A slight narrowness of the black stripes is noted in some cases. This difference is too subtle, however, to

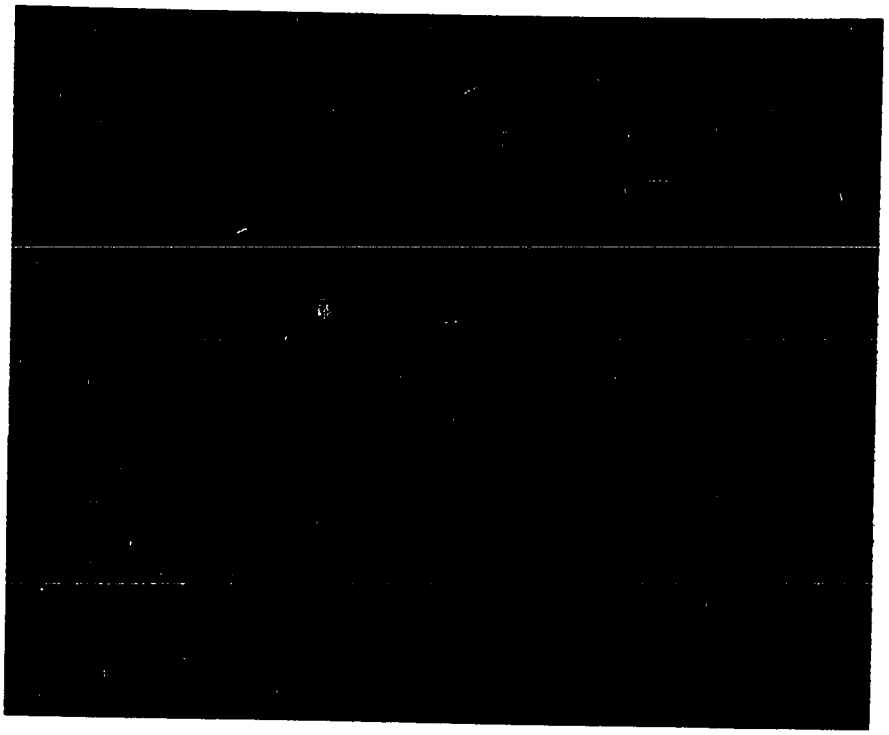


Figure 14. Mh/+ ♂

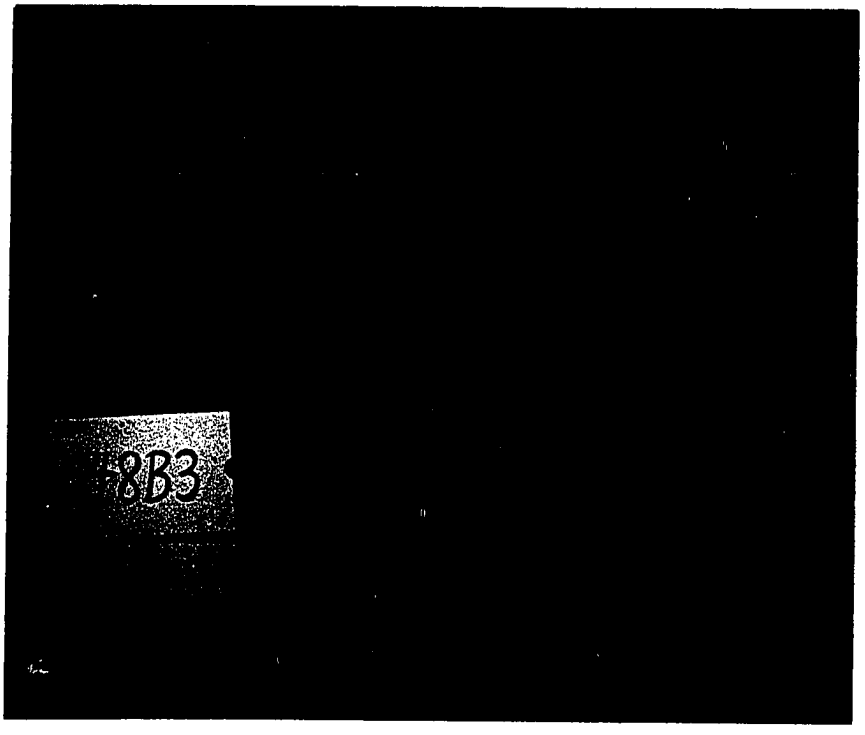


Figure 15. Mh/+ ♀

Table 4. Second backcross matings for segregation of mahogany

Mating #	Mutant parent	Progeny		
		Mh/+	+/+	Total
122	40N1♂	34	17	51
124	40J1♀	20	21	41
130	40M3♂	23	23	46
148	40S2♂	24	24	48
172	40R3♂	16	15	31
174	76G3♂	16	9	25
194	40X2♀	8	7	15
Total		141	116	257
Expected		128.5	128.5	

Pooled chi-square = 2.44
Tabular chi-square (df = 1; .05 level) = 3.84

Table 5. Matings of mahogany heterozygotes for homozygotes

Mating #	Sire	Dam	Progeny			
			Mh/Mh	Mh/+	+/+*	Total
212	40S2	122E4	15	26	9	50
227	40S2	122E3	4	2	0	6
228	40S2	148B3	2	4	5	11
230	40S2	148B2	1	1	1	3
231	40S2	148C13	5	3	5	13
Total			27	36	20	83
Expected			20.75	41.50	20.75	

Pooled chi-square = 2.64
Tabular chi-square (df = 2; .05 level) = 5.99

*Both sexes represented.

permit classification of the chicks. The typical adult male (Figure 16) is a deep red with black remaining in the flights and tail, similar to the Rhode Island Red. The typical female (Figure 17) is essentially a deep red, but remnants of the wild-type stippling still persist.

Because the homozygotes are a deep red in color, I call this mutant mahogany and give it the symbol Mh.

Dilute

The typical dilute heterozygote chick shows a somewhat narrow striping with a paleness evident in the red areas of the pattern. The adult females vary (Figures 18 and 19) but are generally poorly stippled and pale. The paleness is especially evident in the breast. There is a reddish cast in the wing bows. The adult male heterozygote looks like wild type. In some instances, however, the male type may show some red in the breast. Table 6 shows the segregation of dilute and wild type in backcrosses to the wild type. Because the adult males show no phenotypic effect, and because the hens used in these tests were poor layers, the number of birds in each class is relatively small.

Matings of dilute heterozygotes together were equally disappointing. Birds segregating in backcrosses as dilutes from mutant parents carrying more than one factor sometimes

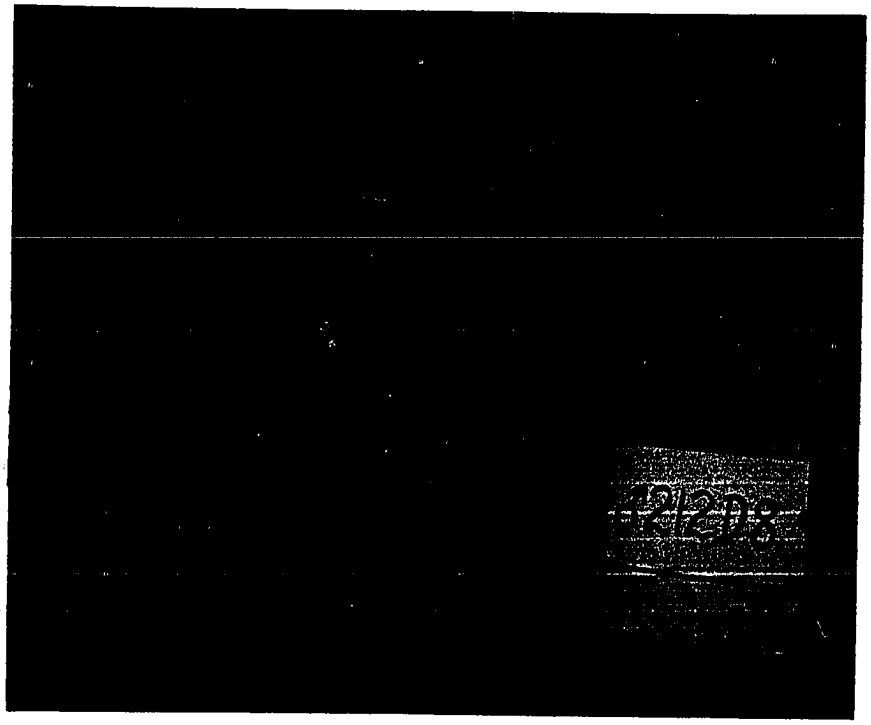


Figure 16. Mh/Mh ♂

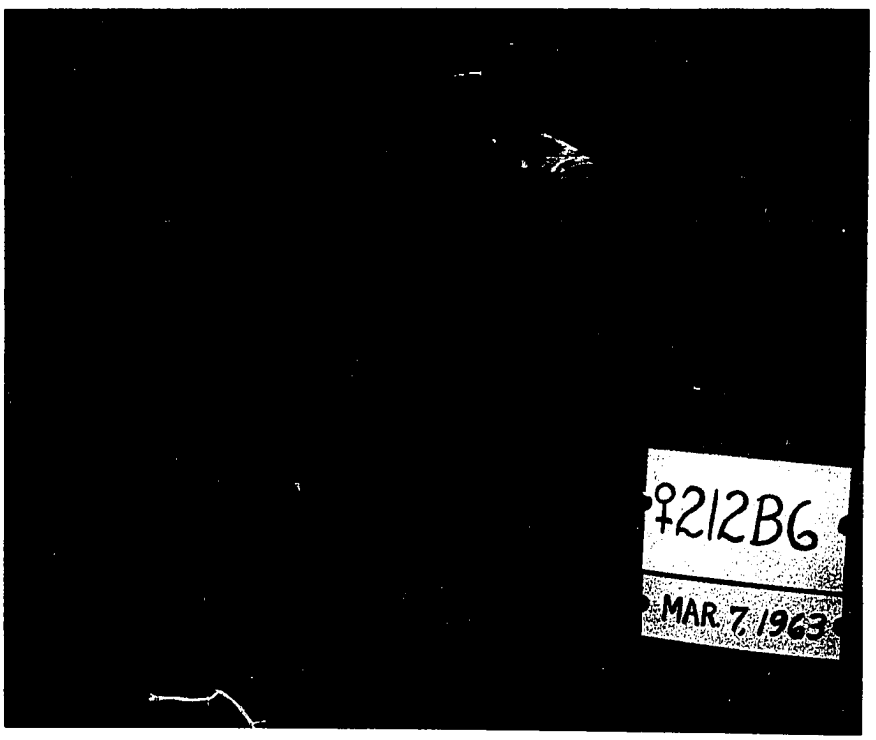


Figure 17. Mh/Mh ♀

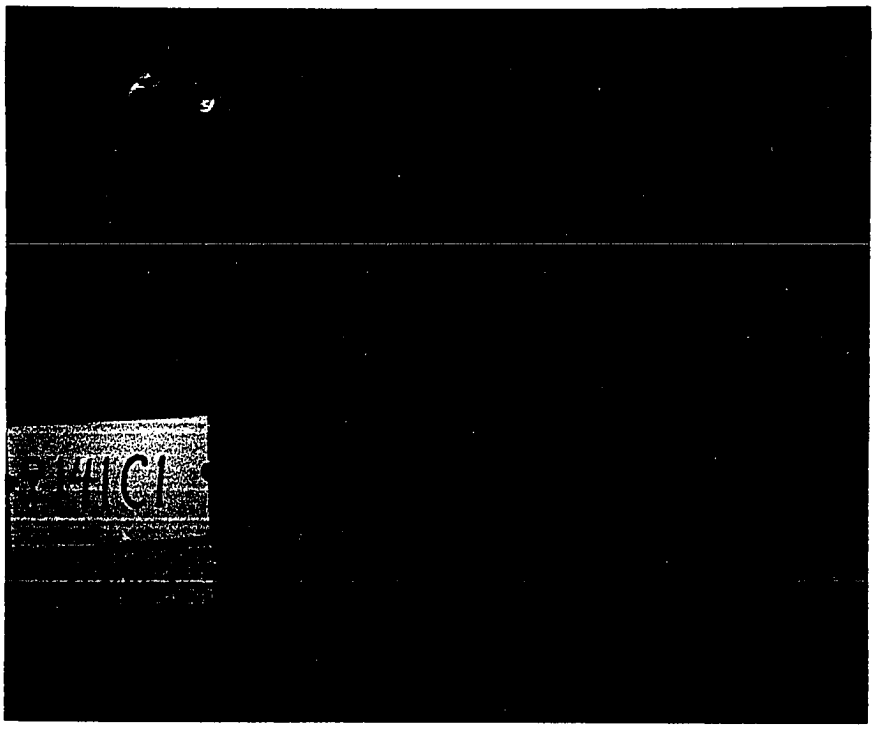


Figure 18. Di/+ ♀

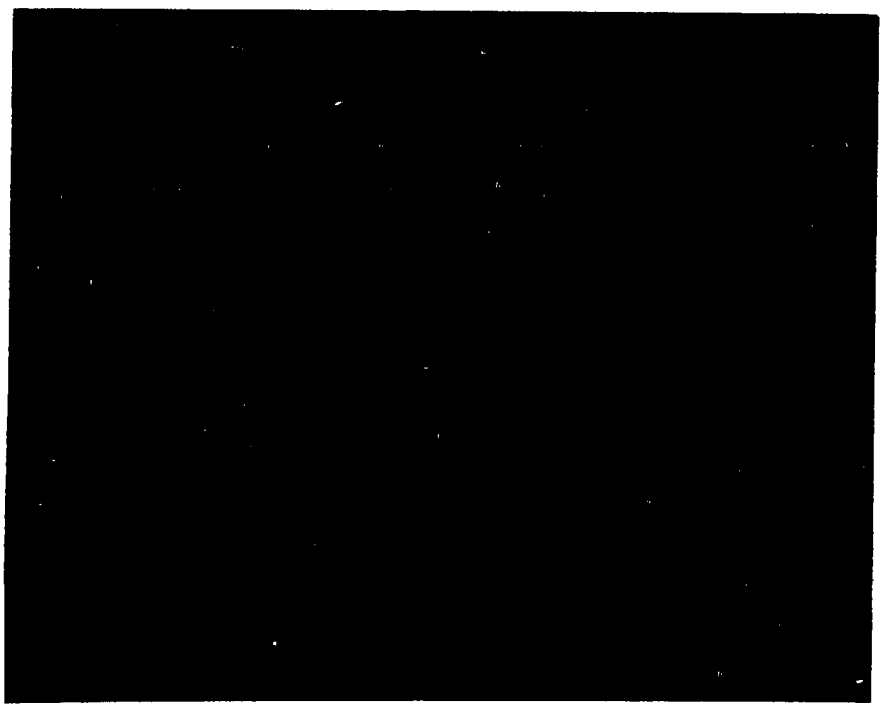


Figure 19. Di/+ ♀

Table 6. Second backcross matings for segregation of dilute (females only)

Mating #	Mutant parent	Progeny		
		Di/+	+/+	Total
109	54B1♂	8	5	13
119	54C3♀	3	7	10
145	54K2♀	3	1	4
161	76D3♀	6	7	13
Total		20	20	40
Expected		20	20	

had to be used. This fact makes the matings less meaningful as it is possible that one of the parents may have been carrying another factor, as well as dilute. Matings of dilute heterozygotes produced three classes of progeny approximating a modified 1:2:1 ratio. Because male heterozygotes look wild type we would expect the following: 2 dilute homozygotes (both ♂ and ♀) : 2 dilute heterozygote females : 1 wild type female : 3 wild type males (see Table 7).

The typical dilute homozygote chick has narrow, pale striping (see Figure 11). Sometimes the median back stripe is shortened. The typical adult male (Figure 20) is pale red. The typical adult female is quite pale. This is especially evident in the red areas of the plumage. Both

Table 7. Matings of dilute heterozygotes for homozygotes

Mating #	Sire	Dam	Progeny				
			Di/Di	Di/+♀♀	+/+♀♀	"+"♂♂*	Total
207	129F1	141C1	7	10	1	4	22
233	145B1	161D6	0	3	2	2	7
236	145B1	170C7	3	0	2	4	9
Total			10	13	5	10	38
Expected			9.50	9.50	4.75	14.25	

Pooled chi-square = 2.60
Tabular chi-square (df = 3; .05 level) = 7.81

*See text.

heterozygotes and homozygotes have reduced melanin pigmentation in the shanks and feet.

Because both the homozygous and heterozygous females exhibit pale, faded plumages, I call this mutant dilute and give it the symbol Di.

Champagne blonde

This phenotype did not manifest itself in the first backcross. Two second backcross males, 115D1 and 127C2 from different dams were the only individual segregates of this type. Because it did not segregate as freely as the other three mutants, I would suggest that it is probably linked

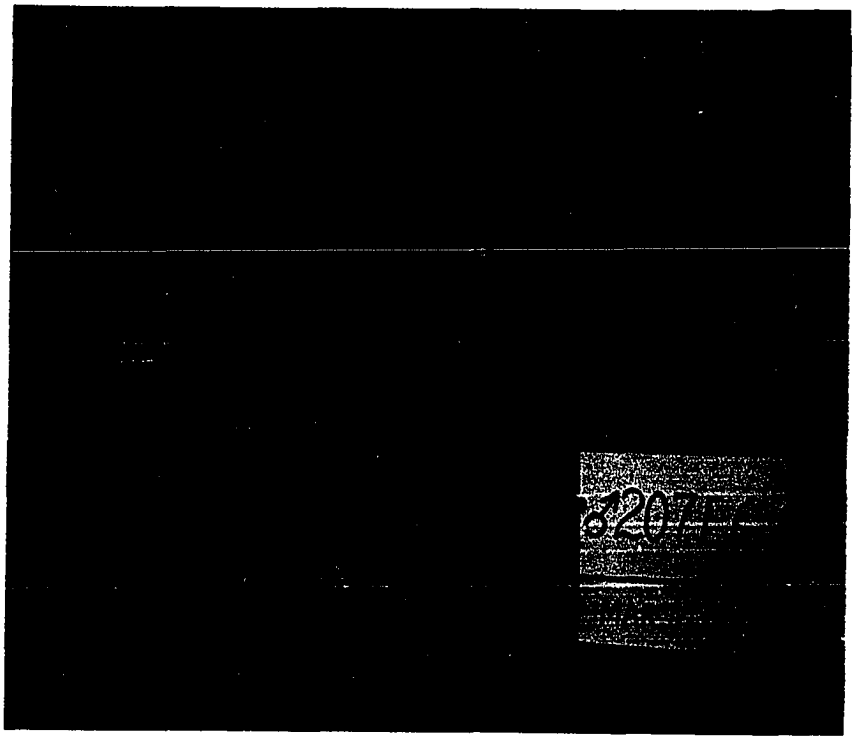


Figure 20. Di/Di ♂

with one of them. The typical champagne blonde heterozygote chick down pattern is wild type. The adult male (Figure 21) is like the "golden duckwing" of the fancier, with a light buffy hackle with some of this buffiness also evident in the tips of the saddle feathers. The adult female (Figure 22) shows almost "silver" phenotype, with very light buff replacing the red areas of the plumage. Apparently only the red areas are affected. A backcross of the blonde heterozygote to the wild type segregated clearly (see Table 8).

Because this type was not found until the second backcross, the homozygote has not yet been obtained. The test

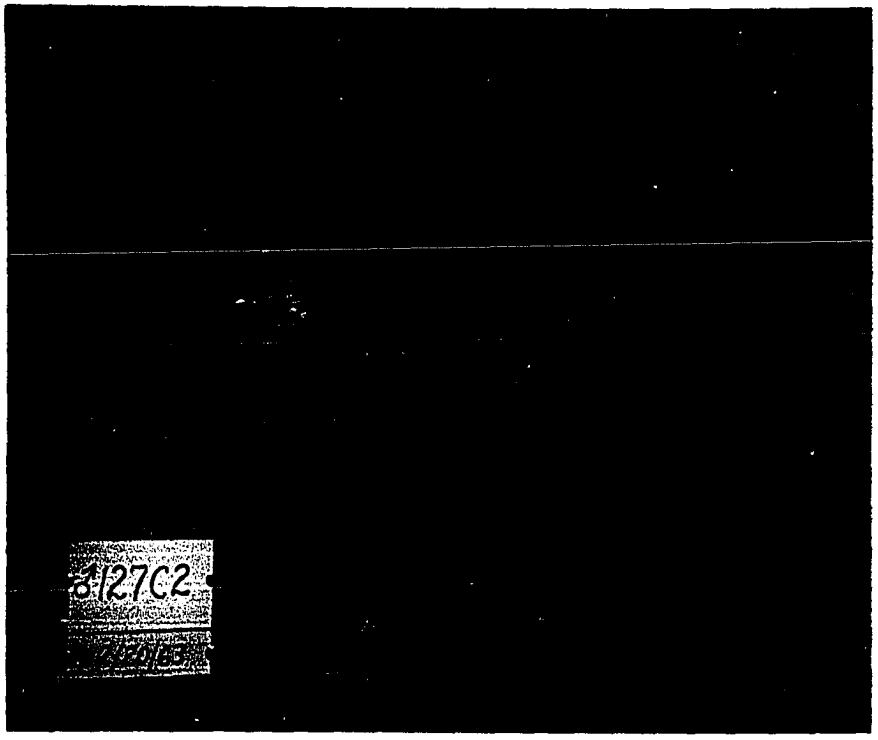


Figure 21. Cb/+ ♂

Figure 22. Cb/+ ♀

Table 8. Third backcross mating for segregation of champagne blonde

Mating #	Sire	Progeny Cb/+	Progeny +/+	Total
203	127C2	13	25	38
Expected		19	19	

Chi-square = 3.78
Tabular chi-square (df = 1; .05 level) = 3.84

for sex linkage also has not been made. This mutant does somewhat resemble sex-linked Silver (S), to which it might be related.

Because this mutant lightens the red areas of the plumage, I call it champagne blonde and give it the symbol Cb.

Interactions

Since there are four dominant or semi-dominant factors segregating in the first backcross, we would expect a total of six possible combinations of two factors. As noted above, however, the champagne blonde factor is probably linked with one of the other three. Since it did not appear until the second backcross, and then only in two birds, it

is probably a close linkage. This would then give us basically only three, seemingly independent, factors. There would then be three possible two-factor combinations, namely: GrDi, GrMh, and DiMh.

Gr/+Di/+ chicks have somewhat narrow striping, with some buffiness evident in the non-black areas of the pattern. They may or may not have a brown upper beak. Apparently, the dilute factor in some cases inhibits the expression of the brown beak associated with ginger. Adult males of this type may resemble the F_1 type or may be light red with some black remaining in the flights and tail. This light red type sometimes exhibits some black stippling in the breast. The adult female (Figure 23) resembles the ginger

Figure 23. Di/+Gr/+ ♀

heterozygote but is somewhat more buffy in the red areas of the pattern, and shows less of the stippling. Distinguishing between Gr/+ and Di/+Gr/+ females is difficult. Table 9 shows the segregation of these two factors in the second backcross.

Gr/+Mh/+ chicks resemble Gr/+ chicks. The adult females of this type also resemble the Gr/+ type. The adult males, however, are mostly dark red with black remaining in the flights and tail. Table 10 shows the segregation of these

Table 9. Second backcross matings for segregation of dilute and ginger (plumage)

		Progeny						
		♂			♀			
Mating #	Mutant parent	+/+ & Di/+	Gr/+	Gr/+ Di/+	+/+	Di/+	Gr/+ or Di/+ Gr/+	Total
90	18E9♀	2	2	1	1	1	2	9
95	18F9♀	1	4	1	2	0	1	9
102	18H2♀	7	1	3	2	2	7	22
127	66E6♀	12	5	5	7	5	10	44
131	54I2♀	0	1	0	1	1	1	4
141	40O2♀	10	6	2	4	8	3	33
165	40J2♀	5	5	0	6	2	2	20
173	76C2♂	14	7	5	6	13	11	56
	Total	51	31	17	29	32	37	197
	Expected	49.25	24.63	24.63	24.63	24.63	49.25	

Pooled chi-square = 10.10
Tabular chi-square (df = 5; .05 level) = 11.07

Table 10. Second backcross matings for segregation of ginger and mahogany (plumage)

Mating #	Mutant parent	Progeny ♂ +/+	♂ Gr/+	♂ Mh/+	♂ Red (Gr/+ Mh/+)	♀ +/+	♀ Mh/+	♀ Gr/+ or Gr/+ Mh/+	Total
118	40H1♀	9	3	3	6	7	7	9	44
137	40H3♂	8	3	9	8	5	4	15	52
143	40R9♀	2	0	4	2	2	4	3	17
185	76H6♀	1	1	1	3	0	1	3	10
188	63J2♀	2	1	1	4	1	0	1	10
Total		22	8	18	23	15	16	32	133
Expected		16.63	16.63	16.63	16.63	16.63	16.63	33.25	

Pooled chi-square = 8.98
Tabular chi-square (df = 6; .05 level) = 12.59

two factors in the second backcross.

As a test of this interpretation of the behavior of Gr and Mh together, a cross of the separated types was made. Mating 229 (♂ 40S2 Mh/+ X ♀ 138G1 Gr/+) produced only nine progeny that were classified in the adult plumage. One male, however, was of the red type, as expected for the combination.

I have compiled a table, listing matings supposedly showing the segregation of dilute and mahogany in the second

backcross (Table 11). Supposed Di/+Mh/+ chicks resemble Di/+ chicks. The adult males of this type are mostly red with black remaining in the flights and tail. The adult females are paler than wild type with a light reddening effect, especially noticeable in the wing bows.

As can be seen from Table 11, there is an excess of red-type males (Di/+Mh/+) and a deficiency of wild-type males if it is assumed that we are dealing with dilute and mahogany as independently segregating units. This discrepancy could be caused by one or a combination of three factors: (1) Some of the parents of this group, although they look alike, may not be genetically the same. (2) There is also a possibility that some of the birds have been misclassified. (3) Also, dilute and mahogany may be somewhat linked. This is not too likely because the number of wild type females recovered fits the expectation based on the hypothesis of two independently segregating factors. Further investigation of the Di-Mh interaction is needed.

As we have just seen, the red-type males, tested or recovered, are always at least two-factor combinations. I did find matings, however, from red-type males or from females which produced red-type males, which indicated that only one factor was segregating (one-half of the progeny were wild type). However, one-fourth of the males were of the red type, and one-fourth looked like Mh/+. This would suggest

Table 11. Second backcross matings for segregation of dilute and mahogany (plumage)

Mating #	Mutant parent	Progeny ♂ +/+ or Di/+	♂ Mh/+	♂ Red (Mh/+ Di/+)	♀ +/+	♀ * Not-+	Total
115	66E4♀	7	5	11	6	11	40
129	66F1♀	11	13	10	4	14	52
142	63D2♀	1	0	7	1	12	21
151	40Q3♀	1	2	1	1	17	22
160	76G1♀	8	5	0	4	11	28
162	76F3♀	5	1	4	2	8	20
167	76G4♀	2	0	1	1	1	5
170	76D1♂	10	2	12	3	21	48
178	40Z9♀	1	1	1	0	0	3
180	76C1♂	3	3	7	4	6	23
183	63H2♀	3	0	9	5	10	27
193	63K2♀	2	1	0	1	1	5
197	76H10♀	7	0	7	3	14	31
Total		61	33	70	35	126	325
Expected		81.25	40.63	40.63	40.63	121.88	

Pooled chi-square = 28.61
Tabular chi-square (df = 4; .05 level) = 9.49

*Division of this phenotypic class into its components is extremely difficult.

that a dominant modifier was segregating which when in conjunction with mahogany completely restricted the black from the breast. Such a hypothesis fits the data shown in Table 12. Figure 24 shows a male of this type. Figure 25 shows a female. Notice particularly the red in her wing bows.

The linkage suggested for champagne blonde has not been tested further. I do have some evidence which indicates it is linked with ginger, and this is the first factor I would test. In Table 3, for example, the sire of three of the matings is 54F2. In matings 202 and 232 he is mated with his daughters (see mating 138 in Tables 1 and 2. He showed a deeper red in the red areas of his plumage than the Gr/+ male pictured in Figure 9. Figures 12 and 13 show progeny from mating 202 (54F2 X daughter), which I believe to be Gr/Gr. In Figures 26 and 27 are Gr/Gr progeny from mating 192. They are lighter and more buffy looking than those from mating 202. The difference, I believe, is due to the fact that the lighter more buffy birds have the champagne blonde factor, either heterozygous or homozygous. Since 54F2 was unusual in that he was darker than most other ginger heterozygotes, I suggest that he is the result of a crossover which separated the ginger factor from the champagne blonde factor. Another small portion of evidence may be adduced from the fact, already mentioned, that some Di/+Gr/+ males were of the light red type while others were of the F_1 type

Table 12. Second backcross matings for segregation of mahogany and a proposed modifier (plumage)

Mating #	Mutant parent	Progeny ♂ +/+	♂ Mh/+	♂ Red	♀ +/+	♀ * not-+	Total
96	40B1♀	6	3	5	6	1	21
101	40E1♂	4	3	5	6	7	25
103	40D5♀	3	2	4	3	1	13
106	40C6♀	5	2	2	6	6	21
110	40K2♂	13	10	4	16	13	56
120	40I4♀	1	2	2	0	1	6
121	66C3♀	8	2	7	7	10	34
139	40N3♂	14	12	2	21	11	60
146	40T2♀	9	2	4	12	19	46
152	63B1♀	1	0	3	3	1	8
154	76E4♀	3	2	12	8	7	32
177	40Z8♀	4	2	5	6	6	23
Total		71	42	55	94	83	345
Expected		86.25	43.13	43.13	86.25	86.25	

Pooled chi-square = 6.80
Tabular chi-square (df = 4; .05 level) = 9.49

*Division of this phenotypic class into its components is extremely difficult.

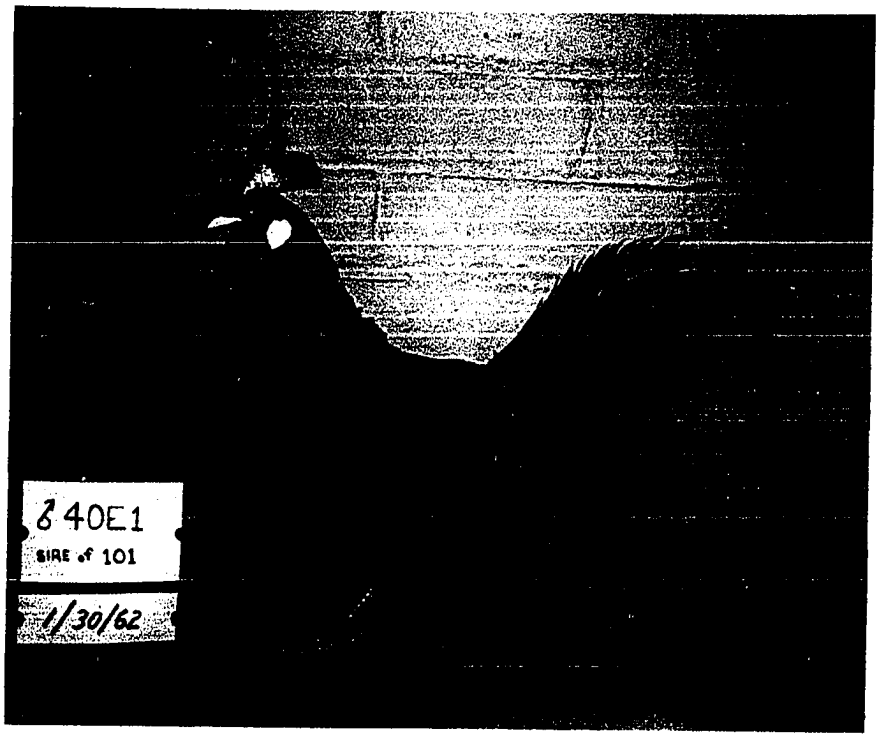


Figure 24. Mh/+ ♂ with modifier

Figure 25. Mh/+ ♀ with modifier

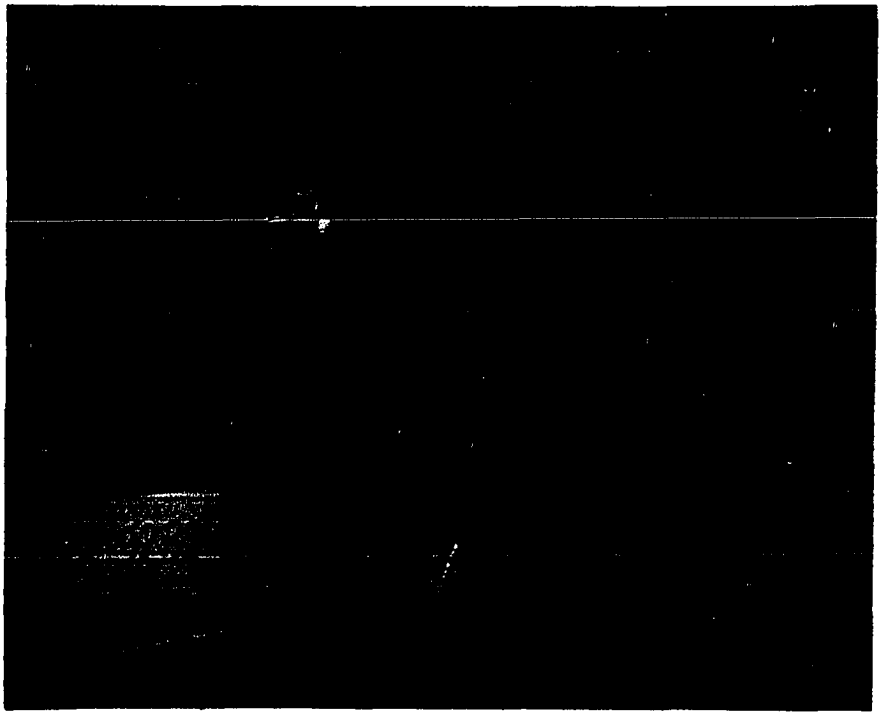


Figure 26. Gr/Gr ♂ with possible Cb

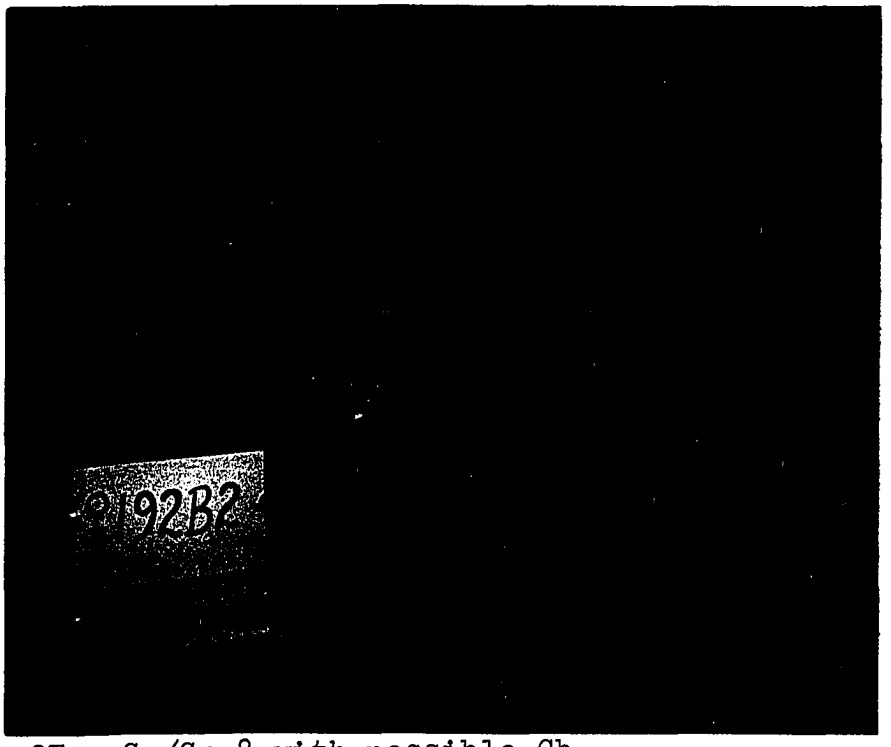


Figure 27. Gr/Gr ♀ with possible Cb

(buffy). The difference again, I propose, can be attributed to the fact that the more buffy males had the champagne blonde factor.

Analysis of the first backcross and F_2 generations

Based on the interactions we have just discussed, we would expect the male phenotypes of the first backcross to be as indicated in Table 13, disregarding the champagne blonde factor because of its proposed close linkage.

Based on these expectations and assuming independent segregation, we can now examine the first backcross data (see Table 14). Again, as in Table 11, we notice that we have an excess of the red or buffy males and a deficiency of wild-type males. This can be caused by two possible factors: (1) Some of the birds have been misclassified. The interactions are possibly not clearly understood. (2) There is some loose linkage involved. This is unlikely because the number of wild-type females recovered fits the expectation based on the hypothesis of three independently segregating factors. In this series of matings we can minimize the possibility that some of the parents are from a different population since all the parents were full-sib F_1 from presumably pure parental types.

Two F_2 matings (52 and 56) produced a total of 381

Table 13. Expected male phenotypic groups based on independent segregation

Genotype[a]	Phenotype
Gr Mh Di *	buffy (F_1 type)
Gr Mh Di +	buffy (F_1 type)
Gr Mh + *	red
Gr Mh + +	red
Gr + Di *	red
Gr + Di +	red or buffy
Gr + + *	Gr/+
Gr + + +	Gr/+
+ Mh Di *	red
+ Mh Di +	red
+ Mh + *	red
+ Mh + +	Mh/+
+ + Di *	+ (?)
+ + Di +	+
+ + + *	+
+ + + +	+

Expected totals:

red or buff = 9/16
+ = 1/4
not +, not red or buff = 3/16

[a] * = assumed dominant modifier of mahogany.

Table 14. Segregation of the first backcross generation (plumage)

Mating #	Mutant parents	Progeny ♂ "+"	♂ red or buffy	♂ not-+ not red	♀ +	♀ not-+	Total
18	4D4♂ 4G2♂	2	17	2	4	19	44
40	4D2♀ 4E1♀	5	36	6	6	64	117
54	4A3♀	5	7	7	2	10	31
58	4G1♀	0	4	1	1	3	9
63	4A2♂	2	11	0	1	15	29
66	4H2♀	0	6	2	0	8	16
76	4K3♀	0	18	2	0	11	31
Total		14	99	20	14	130	277
Expected		34.63	77.85	25.95	17.31	121.17	

Pooled chi-square = 20.65
Tabular chi-square (df = 4; .05 level) = 9.49

progeny which were classified in plumage. Only three wild type males and two wild type females were recovered. These numbers are too small to enable us to draw any definite conclusions, except to note that it points out the fact that as we have seen several factors are required for buff coloration.

Notes

Two "red" breeds were also partially analyzed, Rhode Island Red and Speckled Sussex. Only hens of these breeds were used, to eliminate Id.

A female F_1 (Rhode Island Red X wild type) chick skin is pictured in Figure 6. The first backcross generation from such a female segregated as shown in Table 15.

Table 15. Segregation of the first backcross generation from the Rhode Island Red (plumage)

Mating #	Mutant parent	Progeny ♂ +	Progeny ♂ not +	Progeny ♀ +	Progeny ♀ not +	Total
205	99B5♀	11	14	4	21	50
Expected[a]		6.25	18.75	3.13	21.88	

Chi-square = 5.08
Tabular chi-square (df = 3; .05 level) = 7.81

[a]Based on the assumption of the same three independent factors as in the Buff Minorca.

Not only does the number of factors appear to be the same as the Buff Minorca, but, based on the phenotypes segregating, the Rhode Island Red appears to have the same ginger, mahogany and dilute factors.

From the phenotypes segregating in the F_2 generation (matings 111 and 117) of a Speckled Sussex X Junglefowl cross, it appears that this breed also contains the above mentioned factors. What then is the difference between the red and buff breeds? I would suggest that the champagne blonde factor is responsible for the difference in depth of redness; the modifier of mahogany for the difference in the amount of black. A cross of a Buff Minorca male with a New Hampshire red female (mating 198) produced twelve progeny that were classified in plumage. These F_1 birds were mostly pale red to buff. This indicates the dominance of the factor responsible for the buff coloration. The champagne blonde factor, as we have seen, is a dominant.

It is interesting to note that Kimball (1954) obtained a semi-dominant mutant which he called "red breast (Rb)" from the Cornell Junglefowl flock. It cannot be determined if this mutant is in any way related to any of those mutants isolated from the Buff Minorca. The "dominant" mutants isolated from the buff apparently do not correspond to any genetic types previously identified such as lacing, spangling, etc.

There was noted no obvious correlation between any of the four factors isolated and nutritional requirements, behavior, growth rate, size, or other physiological characteristics.

The "E" Locus

Beginnings

When I began my research program here at Iowa State in the summer of 1960, Dr. Hollander had already made some crosses which indicated that two particular recessive mutants he had were alleles. The origin of speckled head, one of these mutants, is diagrammed in Figure 5. The other mutant was called Wheaten, a color variety of Game bantams which are nearly all yellow as chicks. These recessive types had been crossed and were suspected of being alleles, since the F_1's were not wild type but like the speckled head type. I then proceeded to obtain F_2 and backcross progeny from these individuals to further test their allelic properties. See matings 10 and 45 in Table 18 and matings 8, 9, and 16 in Table 19. No wild type progeny were produced so it was concluded that they were alleles.

In 1955 Dr. Hollander had obtained some indication that the above mentioned Wheaten type and the extension of black factor (E) were also alleles.

Although Morejohn (1955) did not actually perform an allelism test between E and Wheaten (he called the baby chick pattern "yellowish-white"), he had indirect evidence that they were alleles based on the fact that they were both

allelic to the two other types he investigated. I then proceeded to backcross two F_1 females (E X Wheaten) to Wheaten. See matings 14 and 38 in Table 18. Since no wild type progeny were produced, it was confirmed that E and Wheaten were alleles.

In the Buff Minorca/Junglefowl F_2 I noticed that some stripeless or nearly stripeless chicks were being produced which were not produced in the backcrosses to the Junglefowl. This indicated that probably the buff contained a recessive factor. To test this assumption several of the first backcross generation were crossed to Wheaten stock. The results of these testcrosses are shown in Table 20. Because several of these birds segregated for chick pattern factors, it was suspected that another allele or alleles at this same locus had been found. Two distinct recessive types were found. One of these resembled the Wheaten type. The other type, showing more striping than Wheaten, was further tested and found to be a <u>new</u> allele. See mating 169 in Table 18.

Kimball (1960) reported that the Wheaten phenotype was dominant. One of his sources was the Salmon Faverolle breed. Since our Wheaten type had never shown any dominance, we were puzzled. Salmon Faverolle hatching eggs were purchased and chicks raised and breeding tests begun to show what relationship existed between its Wheaten and the previously mentioned recessive wheaten.

From circumstances such as these emanated a project in which several chick pattern factors, in addition to the ones just mentioned, were tested for allelic and dominance relationships.

Description and source of the mutants

Six mutant types were used. A brief description of each type, and their source or sources, will be given.

Extension of black--See the description of the Black Castilian breed in the MATERIALS AND METHODS section for a description of this type. Any of the several common black breeds is also a good source.

Dominant wheaten--See the description of the Salmon Faverolle breed in the MATERIALS AND METHODS section for a description of this type. Salmon Faverolles are silver as well as dominant wheaten. A breed that according to Kimball (1960) carries dominant wheaten without the silver factor is the Wheaten Cubalaya.

Partridge--See the description of the Partridge Rock breed in the MATERIALS AND METHODS section for a description of this type. This factor is often found in Dark Brown Leghorns, Pencilled or Partridge bantams, and Laced Wyandottes, as well as Partridge Rocks.

Speckled head--The chicks of this type have a

speckled and irregular (usually blurred) head and eye stripe but the back pattern is nearly wild type. The adult male is wild type. The adult female is more coarsely stippled than wild type and has more red and yellow in her plumage. As has already been noted, the origin of Dr. Hollander's stock is diagrammed in Figure 5. Morejohn (1955) had extracted a similar type out of Brown Leghorns, which I feel is the same factor.

Buttercup--The chick pattern is quite similar to that of the Buttercup breed described in the MATERIALS AND METHODS section. The adult males, unlike those of the Buttercup breed, are wild type. The adult females show a barring tendency on a buffy ground color. The barring is especially evident in the back. This type is found in the Buttercup breed and was found to be in the Buff Minorca (see Table 20).

Recessive wheaten--See the description of Wheaten Game bantams in the MATERIALS AND METHODS section. Figure 28 shows an adult wheaten female. This type (called "yellowish-white") was found by Morejohn (1953) to be segregating in his Junglefowl stock. It was also segregating in the Lincoln Park Zoo Junglefowl stock obtained by Dr. Hollander. As we shall see it is also a component of the Columbian pattern and its non-silver counterparts.

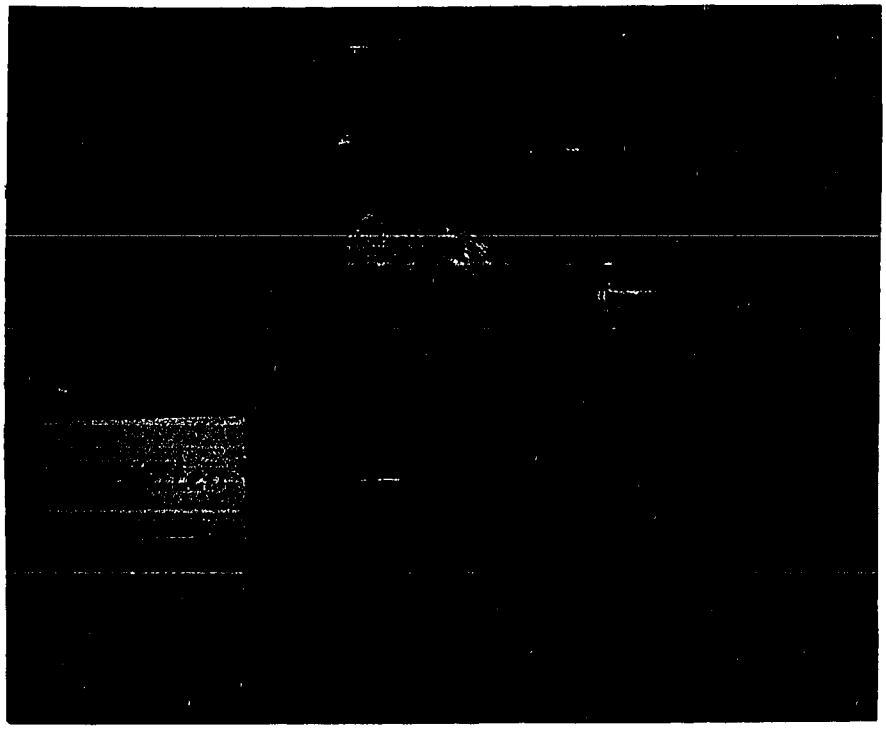


Figure 28. e^{y}/e^{y} (recessive wheaten) ♀

Symbols and identification

The basic symbol for all of these allelic mutants is e(E). This is anticipating that they are all alleles, but it is necessary to present the symbols at this time so that the reader may be able to correctly interpret the tables which will be presented. Capital letters indicate that the mutant is dominant or semi-dominant. Lower-case letters indicate that the mutant is recessive.

E - The extension of black factor has already been

discussed in the REVIEW OF LITERATURE section.

e^{Wh} - Kimball (1960) symbolized the dominant wheaten phenotype as Wh. This symbol is kept but is used as a superscript of e.

e^{p} - Morejohn (1955) used the symbol e^{b} for this phenotype. Since it is characteristic of partridge breeds rather than the Brown Leghorn from which he extracted it, I suggest that the first letter, p, of this breed type be used.

e^{s} - Morejohn (1955) extracted and symbolized this speckled head factor.

e^{bc} - This factor, buttercup, because it is one of the mutants of the Buttercup breed, is given this symbol.

e^{y} - Recessive wheaten. Because he symbolized this e^{y}, "yellowish-white" down, Morejohn (1955) was apparently unaware that it was a widely known characteristic color variety. Because Morejohn has the precedent, because Drosophila symbolization is not yet widely accepted or used by poultry geneticists (Wh and wh would not be the dominant and recessive alleles in the Drosophila system, but would represent different mutants), and to avoid confusion, I suggest that the e^{y} symbol be retained.

It is understood that the + symbol in all cases represents the wild-type allele.

Tables 16 and 17 show the data which establish the fact that each of these mutants is a single factor and also show their dominance relationships to wild type. Both my own data and data from other investigators are included. These are primarily chick data.

Table 16. Matings identifying E locus mutants (backcrosses)

Parents		Mating number or investigator	Progeny	
			E	+
E/+	+/+	13	60	65
		55	11	5
		98	8	8
		100	19	20
		156	18	19
		189	17	20
		216	22	25
		Kimball (1954a) 2-matings	36	37
		Totals	191	199
			E	+
E/+	e^{y}/e^{y}	Bateson & Punnett (1906)	7	6
			E	+
E/e^{y}	+/+	65	15	20
		67	8	17
		Totals	23	37
			$e^{Wh}/+$	e^{Wh}
$e^{Wh}/+$	e^{Wh}/e^{Wh}	Kimball (1960) 3-matings	(1) :	(1)

Table 16. (Continued)

Parents		Mating number or investigator	Progeny	
			$e^{Wh}/+$	+/+
$e^{Wh}/+$	+/+	Kimball (1960) 3-matings	(1) :	(1)
			+	e^{p}
$+/e^{p}$	e^{p}/e^{p}	Morejohn (1955)	9	14
			+	e^{bc}
$+/e^{bc}$	e^{bc}/e^{bc}	157	15	27
		Kimball (1953b) 2-matings	32	29
		Totals	47	56
			+	e^{y}
$+/e^{y}$	e^{y}/e^{y}	33	7	3
		36	11	4
		41	8	10
		61	0	1
		74	9	6
		78	3	1
		80	6	5
		161	35	22
		162	23	17
		172	17	14
		175	33	34
		176	18	17
		Morejohn (1955)	35	39
		Pease & Cock (1951)	89	67
		Serebrovsky (1926)	28	22
		Totals	322	262
			E	Non-E
$\frac{E}{non\text{-}E}$	$\frac{non\text{-}E}{non\text{-}E}$	Cock & Pease (1951) 3-matings	310	326
		Dunn (1923) 6-matings	99	98
		Lippincott (1923)	16	7
		Serebrovsky (1926)	129	153
		Totals	554	584

Table 17. Matings identifying E locus mutants (F_2 & F_2-like)

Parents		Mating number or investigator	Progeny		
			E	+	
E/+	E/+	224	23	9	
		234	24	7	
		Bateson & Punnett (1906)	58	19	
		Kimball (1952b) 4-matings	244	69	
		Kimball (1954a)	77	21	
		Serebrovsky (1926)	120	44	
		Totals	546	169	
		Punnett & Bailey (Punnett, 1923)	(3)	: (1)	
			E	+	
E/+	$+/e^{y}$	Bateson & Punnett (1906)	20	11	
			E	+	
E/e^{y}	E/+	Bateson & Punnett (1906)	16	7	
			e^{Wh}	$e^{Wh}/+$	+
$e^{Wh}/+$	$e^{Wh}/+$	Kimball (1960)	(1)	: (2) :	(1)
			e^{Wh}/e^{p}	e^{Wh}/e^{y}	+
$e^{Wh}/+$	e^{p}/e^{y}	237	3	6	7
			e^{Wh}/e^{bc}	e^{Wh}/e^{y}	+
$e^{Wh}/+$	e^{bc}/e^{y}	241	2	3	6
			+	e^{p}	
$+/e^{p}$	$+/e^{p}$	Morejohn (1955)	23	8	

Table 17. (Continued)

Parents		Mating number or investigator	Progeny	
			+	e^s
$+/e^s$	$+/e^s$	Hollander (1962, unpublished)	22	10
		24	14	6
		25	17	2
		Totals	53	18
			+	e^{bc}
$+/e^{bc}$	$+/e^{bc}$	135	89	45
		201	43	11
		Kimball (1953b)	66	18
		Totals	198	74
			+	e^y
$+/e^y$	$+/e^y$	111	74	29
		117	55	15
		207	35	22
		210	33	15
		Morejohn (1953)	41	13
		Morejohn (1955)	66	19
		Pease & Cock (1951)	205	71
		Serebrovsky (1926)	13	4
		Totals	522	188
		Bateson & Punnett (1908)	(3) :	(1)
			E	non-E
$\frac{E}{non\text{-}E}$	$\frac{E}{non\text{-}E}$	Asmundson & Milne (1930)	36	15
		Davenport (1909)	61	25
		Hurst (1905)	88	31
		Kimball (1952b)	70	20
		Lippincott (1923)	50	13
		Totals	305	104

Allelism test

Using the various tests outlined in the MATERIALS AND METHODS section, each of the above-mentioned mutants was tested with at least one of the other mutants to see if they were alleles. Then following the transposed geometric axiom, mutants allelic to the same mutant are allelic to each other, I concluded that all six of these factors are alleles. The various allelism tests and their results are presented in Tables 18 and 19. Notice that wild-type progeny were not produced by any of the matings except one. This exception will be discussed later.

Dominance relationships

We have already shown the dominance relationships that exist between each of these mutant types and wild type. Since we are dealing with a multiple-allelic series, the dominance relationships between each of them must also be determined. These relationships can be determined by obtaining all the possible combinations of these alleles. This has been done with the exception that the combination of e^{p}/e^{bc} has not yet been determined. These relationships, based on chick patterns are pictured in Figure 29. Notice that both dominant and semi-dominant expressions are represented, with the exception that the e^{s}/e^{bc} combination is

Table 18. Testcross and testcross-like matings showing allelism at the E locus (primarily chick data)

Parents		Mating number or investigator		Progeny	
				E	e^{Wh}
E/e^{Wh}	+/+	104		28	28
		171		23	28
			Totals	51	56
				E	e^{Wh}
E/e^{Wh}	e^{s}/e^{s}	200		8	6
				E	e^{Wh}
E/e^{Wh}	e^{y}/e^{y}	133		53	41
		134		7	13
			Totals	60	54
				E	e^{p}
E/e^{p}	e^{p}/e^{p}	Agar (1924)		12	13
				E	e^{y}
E/e^{y}	e^{y}/e^{y}	14		52	38
		37		9	19
		38		28	32
		48		5	11
		82		6	10
			Totals	100	110
				e^{Wh}	e^{s}
e^{Wh}/e^{s}	e^{y}/e^{y}	94		4	2
				+	e^{p}
$+/e^{p}$	e^{y}/e^{y}	223		26	18

Table 18. (Continued)

Parents		Mating number or investigator		Progeny	
				+	e^{p}
$+/e^{s}$	e^{p}/e^{p}	Morejohn (1955)		23	28
				+	e^{bc}
$+/e^{bc}$	e^{y}/e^{y}	81		6	17
		101		29	33
		120		8	5
		124		27	29
		128		18	19
		139		31	35
			Totals	119	138
				e^{p}	e^{p}/e^{s}
e^{p}/e^{s}	e^{p}/e^{p}	Morejohn (1955)		14	12
				e^{p}	e^{s}
e^{p}/e^{s}	e^{s}/e^{s}	Morejohn (1955)		10	10
				e^{p}	e^{y}
e^{p}/e^{y}	e^{y}/e^{y}	206		23	28
		213		8	15
		Morejohn (1955)		15	21
			Totals	46	64
				e^{s}	e^{y}
e^{s}/e^{y}	e^{y}/e^{y}	10		41	28
		45		9	11
			Totals	50	39
				+*	e^{s}
e^{bc}/e^{y}	e^{s}/e^{s}	209		16	19
		209a		13	6
			Totals	29	25
				e^{bc}	e^{y}
e^{bc}/e^{y}	e^{y}/e^{y}	169		28	26

*e^{s}/e^{bc} acting as complementary alleles.

Table 19. F_2 and F_2-like matings showing allelism at the E locus (primarily chick data)

Parents		Mating number or investigator	Progeny		
			E	e^{Wh}	
E/e^{Wh}	E/e^{Wh}	Serebrovsky (1926)	84	37	
			E	e^{s}	
E/e^{s}	E/e^{s}	Morejohn (1955)	72	15	
			E	e^{p}	e^{s}
E/e^{s}	e^{p}/e^{s}	Morejohn (1955)	81	33	37
			+	e^{p}	
$+/e^{p}$	$+/e^{s}$	Morejohn (1955)	60	17	
			+	e^{bc}	e^{y}
$+/e^{y}$	e^{bc}/e^{y}	211	13	4	4
			e^{p}	e^{s}	
e^{p}/e^{s}	e^{p}/e^{s}	21	56	20	
		Morejohn (1955)	129	51	
		Totals	185	71	
			e^{p}	e^{p}/e^{y}	e^{y}
e^{p}/e^{y}	e^{p}/e^{y}	215	15	15	11
		Morejohn (1955)	32	50	28
		Totals	47	65	39

Table 19. (Continued)

Parents		Mating number or investigator		Progeny		
				e^s	e^y	
e^s/e^y	e^s/e^y	8		1	0	
		9		56	27	
		16		45	16	
		126		12	3	
			Totals	114	46	
				e^{bc}	e^{bc}/e^y	e^y
e^{bc}/e^y	e^{bc}/e^y	220		3	7	4
		221		7	10	8
			Totals	10	17	12

wild type. This is unexpected and unusual and will be discussed further in the following section. We can conclude then from Figure 29 that the alleles in their order of descending dominance are E, e^{Wh}, +, e^p, e^s, e^{bc}, and e^y.

Most of the work with this E locus has been based on the expression of these alleles in the chick pattern. Work with the adult expressions is difficult for two reasons: (1) all of the adult males look wild type except those with E and (2) the stippling pattern in the females is quite similar for the various combinations of e^p, e^s and e^{bc}. It is difficult or impossible to classify these combinations on the basis of adult phenotype alone. Also, some of the adult

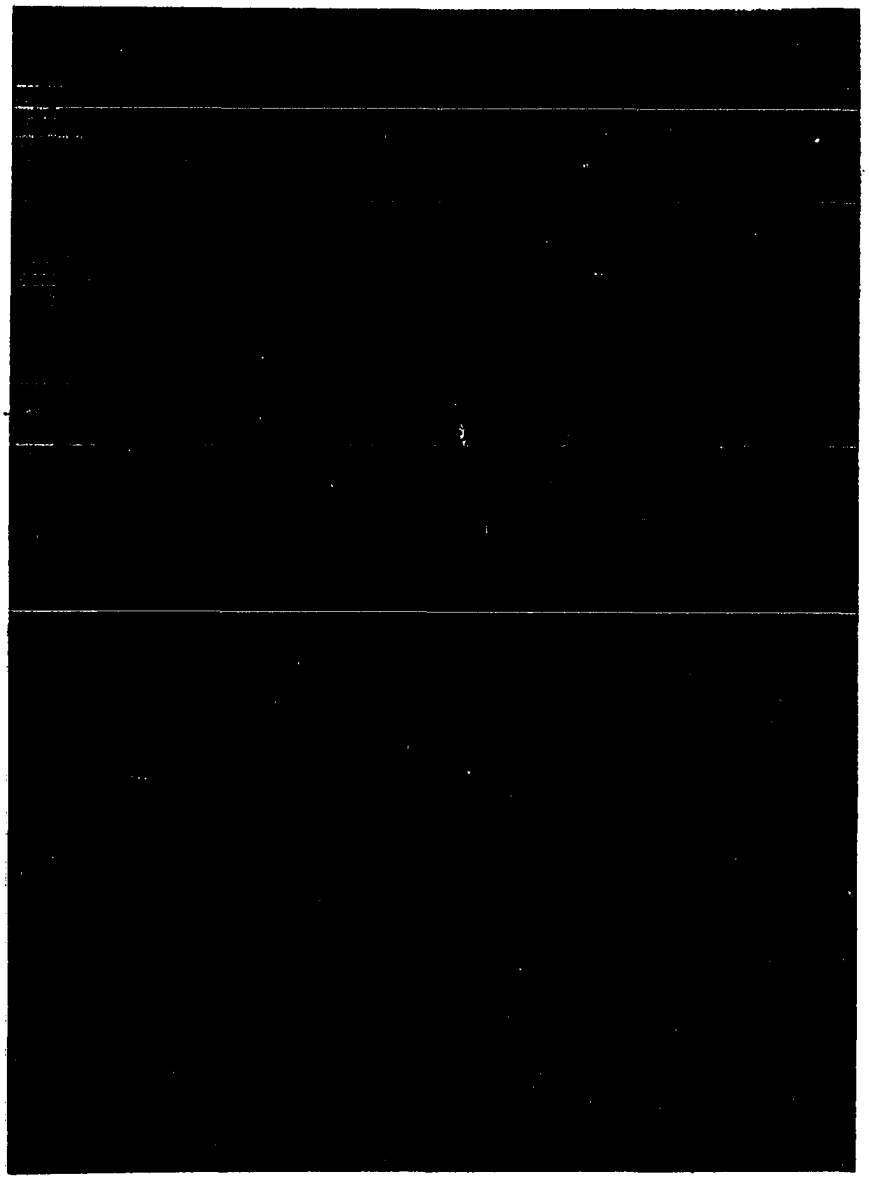


Figure 29. The various combinations of the E alleles (baby chick)

combination phenotypes have not yet been determined.

Complementary alleles

e^s tested as an allele of e^y, and e^{bc} tested as an allele of e^y ∴ e^s and e^{bc} are alleles. I wished to determine which of these two was dominant to the other. When these two were brought together, however, wild-type progeny were produced. This result would ordinarily indicate that they are not alleles, except in the cases of "paramutation," and of complementary alleles in bacterial genetics. Birds of the e^s/e^{bc} genotype have not yet been tested, so it is not known whether e^s and e^{bc} will separate and maintain their identity. The undetermined combination of e^p/e^{bc} might also give information that would indicate what is happening here. Since e^s and e^{bc} together cause a normal (wild-type) phenotypic expression they are here termed complementary alleles.

What is the Columbian pattern?

I hope that the reader has noticed that the Columbian pattern (so-called "e") has not been included in our analysis of the E locus. This is the pattern type characteristic of such breeds as the Buff Minorca, Rhode Island Red, Speckled Sussex and New Hampshire. Its silver counterpart is found in the Light Sussex, and other Columbian

breeds. Both sexes are alike in plumage color.

As we saw in the REVIEW OF LITERATURE section, Cock and Pease (1951) questioned the unifactorial nature of "e." We have seen in the "Buff Minorca Analysis" section that the dominant expression of the Columbian pattern actually consists of three semi-dominant mutants as extracted from both Buff Minorca and Rhode Island Red.

Does the Columbian phenotype also contain a mutant at the E locus? Matings 19 and 20 (F_2 from Buff Leghorn X e^s/e^s) produced 149 chicks. None of the chicks was wild type. This indicated that an E locus mutant was present. As we have already noted the Buff Minorca male used in our analysis was apparently heterozygous at the E locus, probably having the genotype e^{bc}/e^y as indicated from the data in Table 20. F_1 birds from Rhode Island Red X Junglefowl crossed with e^y/e^y stock produced e^y chicks. (See matings 175 and 176 in Table 16.) An F_2 from a Speckled Sussex X Junglefowl cross also produced e^y chicks (see matings 111 and 117 in Table 17). From this we can conclude that the E locus mutant present in the Rhode Island Red and Speckled Sussex is the <u>recessive</u> e^y, which does <u>not</u> affect the male phenotype.

We can conclude then that the Columbian pattern consists of more than one mutant and probably of three semi-dominants and one recessive.

Table 20. First backcross (Buff Minorca study) crossed with recessive wheaten stock (baby chick)

Mating #	Mutant parent	Progeny		
		e^+	Very narrow stripe (e^{bc})	Wheaten (e^y)
80	18F5♂	6	0	5
81	18D11♂	6	17	0
101	40E1♂	29	33	0
110	40K2♂	59	0	0
120	40I4♀	8	5	0
122	40N1♂	51	0	0
124	40J1♀	27	29	0
128	40M10♀	18	17	0
129	66F1♀	55	0	0
139	40N3♂	31	35	0
140	40S1♀	34	0	0
141	40Q2♀	48	0	0
161	76D3♀	35	0	22
162	76F3♀	23	0	17
172	40R3♂	17	14	0

Tested heterozygous for a recessive = 10/15
Tested heterozygous for very narrow stripe (e^{bc}) = 7/10
Tested heterozygous for wheaten (e^y) = 3/10

Some interactions of the E locus alleles with other mutants

Can other loci affect the expression of E? A Rhode Island Red-like male, a segregate out of some crosses involving the Dark Fayoumi, Rhode Island Red and Junglefowl (made by Lewis Smith at Iowa State University Poultry Farm) was crossed with the Junglefowl. Surprisingly, approximately one-half of his chicks were black (E). See mating 13 in Table 16. Similarly, a female (55D1) which was one-fourth Buff Minorca and who also had a black female in her pedigree gave approximately one-half black (E) chicks when crossed with the Junglefowl. See mating 156 in Table 16. She was buffy-brown as a chick. Her adult plumage was basically buff with a sprinkling of black stippling in her back, wings and tail.

A mahogany (?) male was crossed with E/E Black Castilian females. The chicks were black with a tinge of brown. The F_1 adult males were mostly black with some yellow evident in the hackle. The F_1 progeny were mated together for an F_2. Approximately three-fourths of the chicks were black or chocolate-colored (E). See mating 234 in Table 17. This chocolate type is pictured in Figure 30.

Two F_1 females from a Junglefowl ♂ X Silver Spangled Hamburg bantam ♀ cross were backcrossed to the Junglefowl. These also produced approximately one-half black or

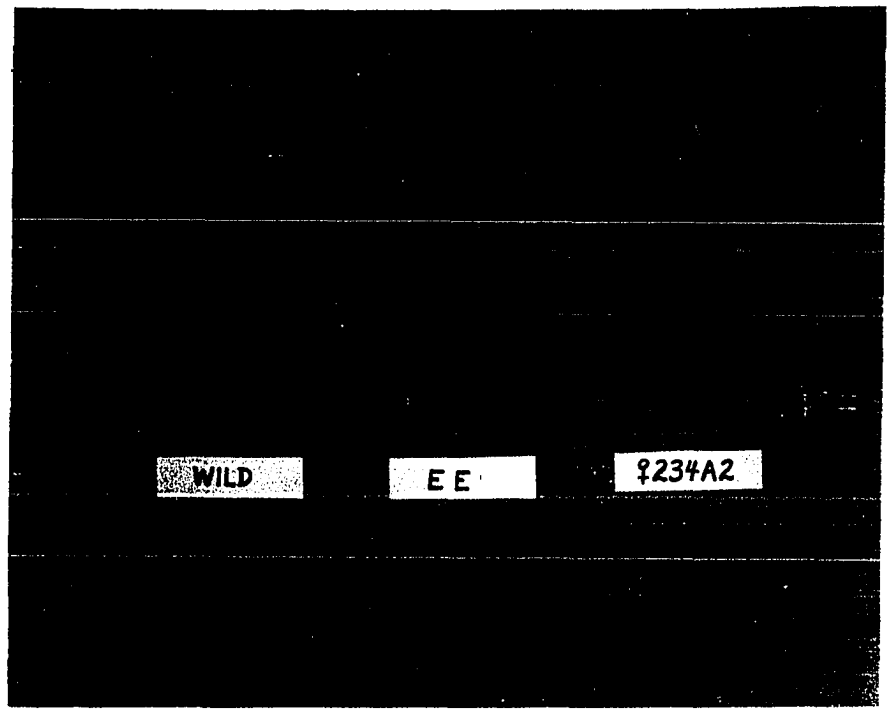


Figure 30. +, E, modified E

chocolate chicks. See mating 156 in Table 16. From these four cases we can conclude that certain mutants can interact with E and mask its usual blackening effect. The Silver spangled Hamburg, the Sebright, the dark Fayoumi, and probably the Campine are examples of modified E expression.

In 1959, Dr. Hollander discovered that recessive wheaten (e^{y}) and recessive white (c) in the double homozygote produced an albino-looking effect. The plumage was white and the eyes were pink. Our original Faverolle male was recessive white as well as dominant wheaten (e^{Wh}). He did not have

pink eyes. Thus, we see that knowledge of this interaction allows us to be able to distinguish between these two similar looking E alleles (e^{y} and e^{Wh}). We also have evidence that the combination of e^{bc}/e^{y} with recessive white gives a similar "pseudo-albino" phenotype.

Linkage of the E locus

We have already noted that the F_2 from the Speckled Sussex X Junglefowl cross segregated for recessive wheaten. The "speckling" effect was recessive, and is probably "mottling," mo, since crosses of Speckled Sussex X Ancona (mottled) are like Anconas in color. The combined F_2 segregation data have a pronounced excess of the mottled wheatens. The results are shown in Table 21. From these data we conclude that apparently the E locus and the mottling factor mo are linked. The indicated cross-over value is about 26%.

Table 21. F_2 from Speckled Sussex X Junglefowl

Mating #	++	+mo	e^{y}+	e^{y}mo	Total
111	46	13	6	10	75
117	39	7	4	6	56
Total	85	20	10	16	131

Pooled chi-square = 18.67
Tabular chi-square (df = 3; .05 level) = 7.81
Estimated crossingover (based on Immer's tables, 1930) = 26% ± 4.6% (standard error)

SUMMARY OF PART I

The Columbian pattern has been shown to be multifactorial. Four semi-dominant factors have been isolated from the Buff Minorca, namely: ginger (Gr), mahogany (Mh), dilute (Di), and champagne blonde (Cb). Gr and Cb are probably closely linked. A dominant reddening modifier of mahogany has also been indicated.

The known alleles at the E locus have been elucidated and symbolized. They are, in order of descending dominance: E, e^{Wh}, +, e^{p}, e^{s}, e^{bc}, and e^{y}. It has also been demonstrated that the blackening expression of E can be masked by certain mutants. Evidence is presented that the E locus and the mottling factor (mo) are linked.

PART II. PHYSIOLOGICAL GENETICS

REVIEW OF LITERATURE

Mutant Autonomy Studies

Grafting is a classical means of analyzing the problem of tissue difference, both those resulting from the normal processes of differentiation and those resulting from genetic change. In regard to color and pattern, such studies have focussed attention on the pigment cells, or melanocytes (melanophores).

Beadle and Ephrussi (1936) grafted imaginal discs from one type of larva to another in Drosophila. They stated (p. 228):

> As a beginning in the study of the differentiation of eye pigment of implanted eyes, it is desirable to know how many eye color mutants are autonomous in their pigment development when implanted in wild-type hosts.

Examples from the above paper are as follows:

> mutant, v, vermilion, grafted to wild type ⟶ wild-type pigmented graft. ∴ v is not autonomous.
>
> mutant, w, white, grafted to wild type ⟶ white graft. ∴ w is autonomous.

The interpretation of autonomy here applies to the mechanism by which the genetic change manifests itself. Basically autonomy indicates that the difference is not mediated by diffusible agents, but rather is under intracellular control.

Many experimental embryologists, apparently unaware of the prior use by Beadle and Ephrussi (1936), have applied the same term to graft results where no genetic difference is involved. Rawles (1948, p. 399) stated:

> While the large body of data agree in showing that the genotypic constitution of the melanophores is the controlling factor in phenotypic expression of color and pattern, it is also clear that the migration of precursor melanophores into the feather germ and their differentiation and arrangements into definitive patterns are not autonomous but influenced to a great extent by a variety of extrinsic factors.

Later she completely confounded the interpretation as she says (Rawles, 1960, p. 230):

> Although it is well established that the genetic composition of the pigment cells is basically responsible for the expression of color and pattern in plumage, it cannot be supposed that the migrations of melanoblasts into the feather primordia or their differentiation and organization into distinctive patterns are autonomous.

Similarly, Hörstadius (1950, p. 85) remarked:

> We have seen that melanoblasts from any region produce a colour and pattern characteristic of the donor. In spite of this, the melanoblasts are not autonomous in determining the pattern. They are also subject to extrinsic factors, such as influences from the feather-germ and from hormones.

These are just examples of a widespread misinterpretation of what grafting experiments reveal. Grafting experiments do not give new information regarding processes which the donor and host have in common. Therefore it is

pointless for these workers to discredit autonomy by referring to fundamental factors necessary for both mutant and wild-type expression.

In effect, however, these various investigators have tested the autonomy of several genotypes of fowl melanoblasts by their methods. In order to be a valid autonomy test, the tissue in question must be removed from donor influences. Danforth and Foster (1929) first demonstrated the autonomy of various pigment types in the fowl by grafting pieces of skin from one breed type onto that of another breed type. This in itself does not necessarily demonstrate autonomy because the donor melanoblasts are still under the influence of their own skin. At the edges of these grafts appear, however, feather chimeras, caused by the migration of pigment cells from the graft into the host's feather follicles. Because these individual host feathers have donor coloration, autonomy is shown.

Willier, Rawles and Hadorn (1937) developed another method; that of grafting embryonic skin into the wing bud of a three-day-old host embryo. Histologic studies showed that the skin, as a tissue, lost its organization and thus the resultant pigmentation was indeed due to the autonomy of the grafted pigment cells. Variations of this method include: (1) grafting wing-bud mesoderm minus the epidermis (Watterson, 1938); (2) grafting wing buds to the body wall

(Eastlick, 1939a); (3) grafting melanoblasts from tissue cultures (Dorris, 1940); and (4) grafting melanoblasts from regenerating adult feather germs (Foulks, 1943).

A more elaborate method, developed by Willier and Rawles (1944) involved two hosts. A donor wing bud is isolated from the donor prior to its invasion by the donor melanoblasts. This wing bud is then placed in the coelom of host #1. If host #1 is a pigmented breed, and if the coelom wall-graft connection is good, melanoblasts will enter the graft. Later, the pigmented skin from this graft may be transplanted to a newly-hatched chick, host #2, for study into adult life.

Still another method consists of injecting suspended melanoblast-bearing tissue into the extra-embryonic yolk-sac circulation of an embryo. The pigment cells are thus disseminated to various parts of the developing embryo where some of the melanoblasts settle and produce donor-specific pigmentation (Weiss and Andres, 1952).

In my own transplantation work, a variation of the Willier, Rawles and Hadorn (1937) method was used. It will be discussed in the section on MATERIALS AND METHODS.

Various genotypes, involving many breed types have been tested. These types are summarized in Table 22.

Particularly notice the autonomy of the E alleles and the buffs and reds.

Table 22. A summary of grafting-autonomy studies

Donor genotype and breed	Hosts	Results	Investigator or results
E			
	Brown Leghorn	a	Danforth (1929a)
Barred Plymouth Rock	Buff Minorca	a	Danforth (1929c)
Black Australorp	Golden Campine	a	Danforth (1935)
Black Minorca	New Hampshire	a	Danforth (1939)
F_1 hybrid	Rhode Island Red	a	Danforth & Foster (1927)
(RIR♂ X BPR♀)	RIR♂ X Bantam♀	a	Danforth & Foster (1929)
		a	Dorris (1938)
		a	Dorris (1939)
		a	Dorris (1940)
		a	Humm (1942)
		a	Rawles (1940)
		a	Willier (1941)
		a	Willier & Rawles (1938a)
		a	Willier & Rawles (1938b)
		a	Willier & Rawles (1938c)
		a	Willier & Rawles (1940)
e^+			
	Barred Plymouth Rock	a	Danforth & Foster (1929)
Brown Leghorn	Black Minorca	a	Eastlick (1939a)
Red Junglefowl	Buff Minorca	a	Eastlick (1939b)
	New Hampshire	a	Eastlick (1939c)
	White Leghorn	a	Trinkaus (1948)
		a	Trinkaus (1950)
		a	Trinkaus (1953)
		a	Weiss & Andres (1952)
		a	Willier (1941)
		a	A=25 P=9 PH=3
		a	A=19 P=1 PH=0 (buff host)

Key:
a = autonomy
- = non-autonomy
Personal results: A = # attempts
P = # pigmented
PH = # pigmented that hatched

Table 22. (Continued)

Donor genotype and breed	Hosts	Results	Investigator or results
e^{p} Partridge Rock	White Leghorn	a	A=62 P=18 PH=7
e^{s} ? (from e^{s}/e^{s} X e^{y}/e^{y})	White Leghorn	?	(trace of darkish pigment) A=12 P=1 PH=0
e^{bc} Buttercup	White Leghorn	a	A=77 P=10 PH=2
e^{y} Wheatens	White Leghorn	a	A=47 P=4 PH=1
	E/+	a	A=14 P=1 PH=1
Buffs	Barred Plymouth Rock	a	Danforth (1935)
Buff Leghorn	Black Jersey Giant	a	Willier (1941)
Buff Minorca	Black Minorca	a	Willier & Rawles (1938b)
Buff Orpington	Brown Leghorn	a	Willier & Rawles (1938c)
	F_1 hybrid	a	Willier & Rawles (1940)
	New Hampshire	a	A=52 P=7 PH=2
	Rhode Island Red	-	A=20 P=0 PH=0 (Buff Minorca host)
	White Leghorn		
	White PR		
	White Silkie		
	White Wyandotte		

Table 22. (Continued)

Donor genotype and breed	Hosts	Results	Investigator or results
Reds	Barred Plymouth Rock	a	Dorris (1939)
New Hampshire	Black Australorp	a	Eastlick (1939b)
Rhode Island Red	Black Minorca	-	Eastlick & Wortham (1946a)
	Brown Leghorn	a	Humm (1942)
	Buff Minorca	a	Rawles (1940)
	F_1 hybrid	a	Rawles (1942)
	White Leghorn	a	Rawles (1945)
	White PR	a	Willier (1941)
	White Silkie	a	Willier & Rawles (1938a)
	White Wyandotte	a	Willier & Rawles (1938b)
		a	Willier & Rawles (1938c)
		a	Willier & Rawles (1940)
		a	Willier, Rawles & Hadorn (1937)
		a	A=16 P=2 PH=0
Golden Campines	Barred Plymouth Rock	a	Danforth & Foster (1929)
Silver Campines	White Leghorn	a	Nickerson (1944)
	White Silkie		
I	Barred Plymouth Rock	a	Danforth (1939)
White Leghorn	Black Australorp	a	Danforth & Foster (1929)
	Black Frizzle	a	Dorris (1939)
	Black Minorca	a	Eastlick (1939a)
	Brown Leghorn	a	Eastlick (1939b)
	Buff Minorca	a	Landauer & Aberle (1935)

Table 22. (Continued)

Donor genotype and breed	Hosts	Results	Investigator or results
	F_1 hybrid	a	Rawles (1940)[a]
	Golden Campine	a	Willier & Rawles (1938a)[a]
	New Hampshire	a	Willier & Rawles (1938b)[a]
	Rhode Island Red	a	Willier & Rawles (1938c)[a]
	White PR[b]	a	Willier & Rawles (1940)[a]
	White Wyandotte[b]	a	Willier, Rawles & Hadorn (1937)[a]
c	Barred Plymouth Rock	a	Eastlick & Wortham (1946a)
White Plymouth Rock	Black Minorca	a	Eastlick & Wortham (1946b)
	Buff Minorca	a	Eastlick & Wortham (1946c)
	Buff Orpington	a	Weiss & Andres (1952)
	F_1 hybrid	a	Willier (1941)
	New Hampshire	a	Willier & Rawles (1938b)
	Rhode Island Red	a	Willier & Rawles (1938c)
	White Leghorn[b]	a	Willier & Rawles (1940)

[a]These authors had negative tests on the BPR, F_1, NH, and RIR hosts.

[b]Authors claim that they can distinguish types here. At any rate, a clear test is doubtful.

Note: Many experimenters have used I (dominant white) and/or c (recessive white) breeds. Grafts from pigmented breeds to these types generally produced pigment.

Ideally a mutant should be grafted onto a wild-type host. Many examples in Table 22 are to mutant types. In all cases where autonomy is indicated, however, the host is of a different genotype than the donor for the particular feature in question. Apparently these combinations permit valid deductions concerning autonomy. As a matter of expediency the majority of hosts were of the white breeds because donor melanoblasts can more effectively migrate and establish themselves in competition with these weaker melanoblasts of the white genotype (Hamilton, 1940).

Valuable information is given, to the geneticist, by autonomy testing. Such investigations reveal much about gene action. If a mutant is autonomous we know that the effect of the difference is not mediated by a diffusible substance but is controlled solely by intra-cellular mechanisms and responses.

Black-red Differentiation

Introduction

All of the genetic factors analyzed in my study affect the relative amounts and location of black and red melanin. At one extreme, black is "extended" (E) to the point of exclusion of red; at the other extreme, buff or red is present

to the exclusion of black. The normal wild type apparently has a balance.

Many experimental embryologists have failed to consider adequately what role the genotype of the materials with which they were working might have on the interpretation of black-red arrangement. Consequently, it is difficult to draw unassailable conclusions concerning normal differentiation processes. Some of these cases will be noted.

I will discuss only the basic principles of this topic, depending largely on the several reviews published.

"Red" and "black" melanocytes differentiate from a common precursor cell

Willier and Rawles (1940, pp. 191-192) stated:

> If two kinds of melanophores [red and black] have already differentiated prior to the formation of the feather germ, it will be necessary to assume the presence of some differential factors controlling their distribution to the red and black areas in the feather vane. It seems more probable that the melanoblasts, at the time of their invasion, are relatively undifferentiated cells. They may have the dual capacity for differentiating into either red or black melanophores, the directions being determined by regional differences in physiological activity within the epidermal substratum of the feather germ. Whether such melanophores are stable and incapable of changing one into the other is an interesting question.

A more definite conclusion was reached by Rawles (1948, p. 400):

> ...the available evidence supports the view that the two types, red and black, differentiate directly from a common precursor cell (melanoblast) rather than the view that red melanophores are merely blacks whose development has been arrested before they reach the black stage. It would appear, then, that melanoblasts of any black-red genotype are potentially capable of differentiating into either black melanophores synthesizing and depositing black, rod-like melanin granules in the feather parts or red melanophores synthesizing and depositing reddish spherical granules: the locus of differentiation, i.e. the epidermal substratum of the particular feather germ or region of the feather germ in which the melanoblast differentiates, determines which of these two potencies is realized. Once a melanoblast becomes fixed (segregated) as to type, black or red, it cannot change to the opposite type. Transitional types have never been observed.

Hamilton (1952, p. 563) agreed:

> In those breeds which have the genetic constitution for red color as well as black (e.g. Rhode Island Red fowl), the melanoblasts are potentially capable of differentiating in either the red or black direction depending upon their milieu. Once they are formed, however, the red and black melanophores are like other differentiated cells in that they are irreversibly fixed, discrete types.

Trinkaus (1953, p. 74) also supported this conclusion:

> These melanoblasts have a dual potency as regards the type of pigment cells they can form; i.e., they may give rise to either black or red melanocytes.

Rawles (1960, p. 230) again emphasized the fact of common origin:

> The two discrete types of melanocytes, red and black, arise from a common melanoblast, "stem cell" potentially capable of differentiating in either one of two directions, depending upon the properties of the

> epidermal substrate in which they differentiate. Transition forms have not been observed.

The conclusion is that "red" and "black" melanocytes differentiate from a common "stem cell." They differentiate after they enter the feather germs. Apparently, once differentiated they can produce only that one kind of melanin.

Environmental factors involved in differentiation

Three environmental situations confront the melanoblast: its relationship to other melanoblasts; its relationship to the immediate tissue environment; and its relationship to the general circulating environment, such as hormones, nutrient supply, etc. What roles are played by these three factors?

Intermelanoblastic relationships These studies have mostly been performed with amphibian material where only black melanophores are found. Twitty (1951) found that melanoblasts isolated in capillary tubes tend to migrate away from each other. Rawles (1955, p. 515) stated:

> Certain experimental studies have shown that pigment cells which have an advantage in age or rate of development are able to inhibit or suppress the differentiation of younger or less rapidly differentiating precursor pigment cells...influences exerted mutually by the pigment cells are of primary importance in their migrations and their arrangement into specific pigmentary patterns.

Similarly, Wilde (1961, pp. 291-292) commented:

> The pertinent data to the present context are that in no case did a single isolated cell undergo differentiation in a microdrop, but that of two cells in a similar drop, one would undergo differentiation....As the number of cells isolated increased (up to fifteen) both rate, degree and numbers of cells differentiating increased.

From this information it can be concluded that melanoblasts tend to affect each other's differentiation.

Melanoblast-tissue relationships From the several reviews already cited and which will be cited below dealing with melanoblasts, there is agreement that: melanoblasts migrate out from the neural crest of the embryo to its various parts. Portions of embryo isolated at various stages of incubation will or will not produce pigment when grafted to the wing bud, chorio-allantois, or coelom of host embryos depending upon whether or not the donor portions had previously been invaded by melanoblasts. Using such grafting techniques it is possible to ascertain at what incubation time melanoblasts reach a certain point in the embryo. Willier (1948, p. 322) stated:

> Up to about 72 hours, the wing bud produces down feathers without pigment, whereas after 80 hours it invariably produces pigmented down feathers (Eastlick, 1939 [c]; Ris, 1941).

Hamilton (1952, p. 564) commented:

> The melanoblasts (presumptive melanophores) can first be recognized in the wing bud of the chick

> embryo of 75 to 80 hours, at which time some of them are entering the epidermis (Watterson). [1942.]

Rawles (1960, p. 216) agreed:

> Information concerning the direction of migration and the time when melanoblasts reach their definitive locations has been obtained chiefly from appropriately designed transplantation experiments with embryos of numerous breeds of domestic fowl (Eastlick, 1939 [c]; Willier and Rawles, 1940; Reams, 1956)....They [melanoblasts] have reached the ectoderm of the wing bud, for instance, by approximately the 80th hour of incubation....

Besides the workers cited by Willier, Hamilton, and Rawles, Fox (1949) performed similar experiments and obtained similar data.

For Willier, Hamilton and Rawles to draw such a general conclusion from these experimenters may be erroneous since they have all, including Fox, tested primarily mutant types rather than normal (wild type) melanoblasts. Genotypic differences may affect the migratory rate of malanoblasts.

The tissue environment in which the melanoblast finds itself guides its migratory pattern. Rawles commented on this (1960, p. 218):

> The fact that the direction and paths of migration are not at random but along preferential routes indicates strongly that the movements of melanoblasts are guided or directed by contact relationships (interactions) with certain other cell strains. The association of cells of distinctly different types presupposes some sort of surface compatibility or affinity.

Perhaps the most concrete example of the effect of

surrounding tissue on melanoblast migration is explained by Rawles (1955, p. 504):

> Certain results obtained from grafting skin in fowl indicate that the invasion of melanoblasts is controlled by the skin and feather germs. When, for example, an area of skin, experimentally deprived of its normal source of pigment cells, is grafted at hatching to a chick host of similar age, melanoblasts from the surrounding regions of the host skin migrate freely into the graft and establish temselves permanently.... Such an invasion of melanoblasts does not take place when an area of normal skin containing its full complement of melanoblasts is grafted similarly (Danforth and Foster 1929). It would appear, then, that invasion does not take place if a state of equilibrium has already been attained between the tissues of the skin and the melanoblasts. This phenomenon has been interpreted by Willier (1948) to mean that a constant ratio has been established between the number of melanoblasts and the cells of the skin. The number of melanoblasts, according to this view, is limited not by a self-limitation of their capacity for multiplication, but rather by the cell community (skin). Such a constant ratio may be temporarily thrown off balance by an active regenerating feather papilla in which special conditions are set up favoring the invasion of some of the melanoblasts from the dermis of its specialized unit, the dermal papilla, into the epidermal region (collar) which gives rise to feather parts. As this invasion of melanoblasts into the regenerating feather parts takes place, other melanoblasts of the dermal regions multiply to restore again the constant ratio. Thus, mechanism is provided for maintaining this constant relationship between the pigment cells and the feather cells throughout the life span of a bird.

On this basis Wilde (1961, p. 285) postulated that the feather germs "attract" melanoblasts:

> In the avian embryo evidence indicates that certain skin organelles such as feather germs may serve as centres to which migrating melanoblasts are attracted. This appears to be distinct from the general location of melanoblasts in the dermis.

In vitro experiments have also produced some interesting results. Trinkaus (1948, p. 152) culturing back skin from 8-day old Brown Leghorn (wild-type) embryos stated: "In all but a few of such cultures large numbers of red melanophores differentiated...." Hamilton (1940, p. 528) using nearly identical methods reported:

> Explants from red breeds [New Hampshire, Rhode Island Red]...rarely differentiated pigment cells containing red melanin.

Although a genetic difference is here demonstrated, Hamilton (1952, p. 563) made the following generalization:

> ...red melanophores, unlike black ones do not commonly differentiate in the usual media of tissue culture.

Dorris (1939) found that Black Australorp melanoblasts differentiated black pigment in vitro; however, Rhode Island Red melanoblasts did not differentiate pigment (red or black) in vitro. She also observed that Black Australorp neural crest grafted to White Leghorn hosts often pigmented dermal structures as well as down plumules. Similar grafts of Rhode Island Red neural crest were never observed to pigment dermal structures; just the down plumules. Based on these findings she suggested that "red" breed melanoblasts were dependent upon the epidermis for differentiation (Dorris, 1939, p. 338):

> Such cells depend for their final color upon factors derived from the host epidermis and probably non-specific in nature, since differentiation occurs both in the normal site and when the tissue is transplanted to hosts of other breeds.

Hamilton and Koning (1952, p. 554) were able to demonstrate this in vitro:

> Red melanophores do not differentiate ordinarily in tissue cultures of embryonic skin from red breeds of fowl when the medium consists of plasma and 10-day embryonic extract. However, if the 10-day extract is partially replaced by an extract of skin and feather germs of 17- to 19-day embryos, then red melanophores differentiate abundantly.

It thus appears that the red breeds require more mature feather follicles and skin for in vitro differentiation of red melanin than the wild type.

Trinkaus (1953, p. 89), again using the Brown Leghorn (wild type), showed that the level of maturity of the epidermis determined the response of the melanoblast to thyroxin and estrogens:

> While Brown Leghorn melanoblasts have the genetic capacity to respond to both thyroid and estrogenic hormone, evidence from tissue culture suggests that this capacity can be expressed only when melanoblasts are differentiating in the presence of the structurally organized epidermis of the feather germ. Moreover this epidermis must have a certain level of organization (maturity) before a hormone effect on melanoblast differentiation is evident. When associated with immature epidermis, the melanoblasts differentiate independently of both hormones and produce a pigment pattern characteristic of down feathers or of juvenile flight or tail feathers, depending upon the tract specificity of the epidermis. In the presence of mature epidermis, melanoblasts respond to these hormones, the

> particular effect on melanoblast differentiation depending on both the tract specific character of the epidermis and the nature of the hormone or combination of hormones involved.

Rawles (1960, p. 218) summarized the situation as follows:

> According to one modern concept of affinitive relations, a cell of a particular type (strain) can become lodged and express its developmental potencies only in "niches" or locations that offer the specific conditions (physical, chemical, physiological) appropriate for one of its particular type. In a nonmatching environment, so to speak, a cell will not thrive.

The evidence just cited suggests that the "niche" for the melanoblast is the feather follicle--skin complex.

Melanoblast-"general" environment relationships

Nutritional factors such as vitamins, minerals, and amino-acids can affect black-red differentiation. Glazener, Mattingly and Briggs (1946, p. 86) reported:

> In three lots of New Hampshire chicks abnormal blackening of the base of the secondaries, primaries and other feathers resulted from feeding a diet deficient in vitamin D.

Decker and McGinnis (1947) found that the fluff of the feathers of Buff Orpingtons darkened when they were placed on a vitamin-D deficient diet. In 1948, Glazener and Briggs, again using New Hampshires, reported that the greater the deficiency of vitamin D the more blackening effect was observed. Blackening was observed in Rhode Island Reds but

not in Buff Plymouth Rocks fed the vitamin-D deficient diet. A low-calcium diet resulted in black feathers; a high-calcium diet coupled with vitamin D deficiency also caused blackening in the New Hampshires. Lillie and Briggs (1947) reported that various levels of folic acid deficiency could cause white or abnormal black regions in feathers of New Hampshires and in F_2's from New Hampshire ♂ X Barred Plymouth Rock ♀. The mechanisms of these nutritional factors is not clear and would warrant further investigation. It is interesting to note that in all cases "red" areas were changed to black, never the reverse.

Disease can affect pigmentation. Juhn (1942, 1954a) reported that Brown Leghorn capons showing symptoms of avian leukosis developed red areas in their normally black breast feathers.

Hormones can affect pigment differentiation. As we have seen in the previous section on "Melanoblast-tissue relationships," a "mature" epidermis is necessary to mediate the action of the hormone. The melanoblast does not respond to the hormone alone.

Juhn, Faulkener and Gustavson (1931) showed that estrogens injected into Brown Leghorn (wild-type) capons induce the deposition of a bar of female (red) coloration in developing breast feathers. Further they stated (p. 105):

> There is a direct relation between the growth rate of male feathers in the Brown Leghorn and the concentration of the female hormone essential to female plumage modification.

Hyperthyroidism and hypothyroidism also affect the coloration of Brown Leghorns (wild type). This has been done by many workers, only those noting growth rates will be discussed. The effect of hyperthyroidism is discussed by Hutt (1930, p. 1):

> ...desiccated thyroid when given to Brown Leghorns... caused a greater production of [black] melanin.

Thiouracil-induced hypothyroidism causes black pigment to be replaced by red (Domm and Blivaiss, 1948).

I will now discuss the relationship of hyper- and hypothyroidism to the growth rates of feathers. One should keep in mind that the above-mentioned pigment changes are simultaneously involved. All these workers used Brown Leghorns (wild type). Domm (1929, p. 228) induced hyperthyroidism and noted:

> All medicated birds revealed accelerated replacement of feathers.

Chu (1938, p. 555) drew a similar conclusion:

> ...the period for feather growth was shortened significantly in the thyroid fed birds....

Greenwood and Blyth (1929) found feather growth slower in completely thyroidectomized birds. Chu (1938, p. 555)

speaking of the period of time necessary for feather growth reported it "...lengthened in the thyroidectomized group." He later comments (Chu, 1940, p. 494):

> Retardation of growth of feathers in the denuded areas was a very pronounced effect of hypothyroidism. In a small number of birds the denuded areas failed to regenerate new feathers up to the end of the experiments.

Blivaiss (1946, p. 99) similarly concluded, speaking of his thyroidectomized birds, "...a marked reduction of feather growth rates..." Thiouracil-induced hypothyroidism also caused retarded growth rates (Domm and Blivaiss, 1944; Domm and Blivaiss, 1946; Domm and Blivaiss, 1948).

We conclude then that increased black melanin deposition and an increased growth rate are associated with hyperthyroidism, while red melanin deposition and a decreased growth rate are associated with hypothyroidism.

Several workers have caused hyper- and hypothyroidism in birds of other breeds than the Brown Leghorn. The cases which affect black-red differentiation are tabulated in Tables 23 and 24.

The general conclusion which can be drawn from these cases is that hyperthyroidism increases the amount of black pigment (or its equivalent) and hypothyroidism increases the amount of red pigment (or its equivalent).

Table 23. Effect of hyperthyroidism on various breeds of fowl

Breed	Effect	Investigator
Campine	blacken	Hornung and Torrey (1927)
Rhode Island Red	blacken	Hornung and Torrey (1927)
F_1 Black Minorca/Buff Leghorn (♂ and ♀)	blacken	Danforth (1933a)
F_1 White Leghorn/Buff Leghorn (♂ and ♀)	whiten[a]	Danforth (1933a)
Silver Dorking	blacken	Emmens and Parkes (1940)
Sebright	blacken (laced border thickens)	Emmens and Parkes (1940)
Duckwing Bantam	blacken	Chu (1940)
F_1 Barred Plymouth Rock/ New Hampshire	blacken	Juhn (1954b)
F_1 Brown Leghorn/ New Hampshire	no effect	Juhn (1954b)
F_1 Brown Leghorn ♂/ Columbian ♀	blacken	Juhn (1954b)

[a]Because dominant white eliminates primarily black pigment, an increase in white here is equivalent to a darkening effect in a bird without dominant white.

Table 24. Effect of hypothyroidism on various breeds of fowl

Breed	Effect	Investigator
Silver Dorking	whiten[a]	Parkes and Selye (1937); Emmens and Parkes (1940)
Barnvelder	redden	Parkes and Selye (1937); Emmens and Parkes (1940)
Sebright	redden (loss of lacing on breast)	Parkes and Selye (1937); Emmens and Parkes (1940)
Duckwing game Bantam	whiten[a]	Chu (1940)
F_1 Barred Plymouth Rock/ New Hampshire	redden	D'Angelo and Gordon (1947); Juhn (1954)
F_1 Brown Leghorn/ New Hampshire	redden	Juhn (1954)
F_1 Brown Leghorn ♂/ Columbian ♀	whiten[a]	Juhn (1954)

[a]Because silver eliminates primarily buff and some red pigment an increase in white is equivalent to a reddening effect in a bird without silver.

Histological Studies

In a rather extensive paper, Bohren, Conrad and Warren (1943) compared whole mounts of web and fluff barbules of the Brown Leghorn (wild type) and of red and buff breeds. Concerning the Brown Leghorn they commented (Bohren et al., 1943, p. 500):

> The Brown Leghorn...feathers were unique in that the granules showed a great variation in size, ranging from rods about 1.5 μ long by 0.5 μ in diameter...; down to almost spherical forms about 0.5 μ in diameter.

Using samples from Red Leghorns, New Hampshires, Rhode Island Reds and Speckled Sussex, they reported (Bohren et al., 1943, p. 490):

> In red areas small spherical granules about 0.5 μ in diameter and of very uniform size were found. Another type of granule somewhat oval in character measuring about 0.7 μ in diameter by 1.0 μ in length was found. The latter were far less numerous than the round granules and correspond in size, shape, and distribution to the granules found in buff feathers.

Investigations of Buff Minorcas, Leghorns, Plymouth Rocks, Cochins and Orpingtons revealed (Bohren et al., 1943, pp. 493-494):

> ...slightly oval granules about 0.7 μ in diameter and 1.0 μ long....The lighter the shade of buff, the fewer the granules and the more restricted their distribution.

MATERIALS AND METHODS

Autonomy Testing

Melanoblast-bearing tissue from various breeds of domestic fowl was grafted to White Leghorn host embryos, or in special instances to Brown Leghorn (wild type), Buff Minorca or E/+ embryos. The source of the donor eggs was the McMurray Hatchery, Webster City, Iowa, and my own genetic stocks. The host eggs were supplied by the Veterinary Medical Research Institute of Iowa State University.

In 1961 I employed the grafting methods of Willier, Rawles and Hadorn (1937). Both donors and hosts were incubated for approximately 68-72 hours at 103° Fahrenheit in a small still-air incubator. A piece of donor head skin was then grafted to the wing bud of the host. Sterile technique was used. The eggs were not turned after the transplantation was completed. My results were poor in that most of the embryos died just prior to hatching. Donor pigmentation was evident in these embryos, but a complete discernment of autonomy required that the juvenile plumage be examined.

Consequently, in 1962, a different method was employed. Donor embryos were incubated as before, but for 96-100 hours. Host embryos were incubated the usual 68-72 hours. The host eggs were opened and sealed after the method of Hilleman

(1942). The wing buds of several donors were severed from the body using very fine watchmaker's forceps and placed in Ringer's solution. These were then forced through a Swinny filter without its asbestos filtering pad. The wing buds were thus broken up into fine pieces by being forced through the fine screen, in the filter. This mixture of Ringer's solution and wing bud cells was centrifuged and the supernatant discarded. The cells and bits of wing bud were then resuspended in a small quantity of Ringer's Solution. To transplant, a portion of this mixture was drawn into a microliter syringe fitted with a number 27 hypodermic needle. The cells were then injected into the embryo usually in the leg bud or wing bud regions. The eggs were left undisturbed for two days and were then placed in a Jamesway forced-draft incubator which automatically turned the eggs. They were treated the same as hatching eggs. Many more of these eggs hatched. Although the proportion of "takes" decreased, I was able to achieve my purpose in obtaining hatched, healthy, grafted chicks.

Melanoblast Migration Timing

Wing buds, of various stages of incubation, were transplanted to the chorio-allantois of 8-9 day White Leghorn embryos, using the method described by Hamburger (1960,

pp. 158-165). The operating window was covered after the operation with a sterile coverslip and sealed with wax, rather than being sealed with masking tape as Hamburger suggests. Using this method, I attempted to compare the migration rates of the melanoblasts of the Brown Leghorn (near wild type) and the Black Spanish (E/E) genotypes. The age (hours of incubation) of each graft was noted. If the graft was pigmented, upon examination 10 days post-grafting, it was assumed that melanoblasts had entered the wing bud at or before the time of grafting. If the graft was not pigmented, it was assumed that the melanoblasts had not yet entered the wing bud at the time of grafting.

It was found in early experiments that 8-day host embryos did not have a sufficiently developed chorio-allantois to withstand the operation. Many of my early attempts failed until I began to use 9-day host embryos.

The typical Junglefowl hatches at 20 days of incubation. This implies that domestic breeds develop more slowly. For this reason, I used Brown Leghorns (wild-type melanoblasts in a domestic breed) as my wild-type representative, as this difference in rate of embryonic maturation might affect the migration rate of the melanoblasts, apart from the differences in the melanoblasts themselves.

Thyroxin Experiments

The breast feathers of three male segregates colored about like Rhode Island Reds, from the Buff Minorca analysis (40K2, 40M4, 40N10), were plucked, and samples saved. When the regenerating feathers were just emerging from the mouth of the feather follicle thyroxine treatment was begun. The birds were fed the thyroxine in gelatin capsules by gavage. L-thyroxine (sodium salt) from General Biochemicals of Chagrin Falls, Ohio was used. 40K2 received one dose of 5 mg. 40M4 received 5 mg./day for two successive days. 40N10 received 5 mg./day for three successive days. Changes in feather pigmentation were noted and sample feathers were plucked and kept for a permanent record.

Fasting Experiments

In order to test whether reduced rate of feather growth could alter pigmentation type, fasting was enforced during regeneration. Only two birds were used: a Junglefowl cock (L-25) and a red pyle cock (L-26). The birds were housed individually in wire-floored battery compartments to prevent obtaining nutrients from litter, and the room temperature was about 85°F.

The left breast feathers were plucked. A transverse

row of follicles located in the middle of the breast tract of each bird was marked with an india-ink tattoo. The growth rate of the feathers from the marked row of follicles was determined by taking measurements at various intervals. Measurements were made in millimeters from the mouth of the follicle to the tip of the feather. The feathers were again plucked and saved for a permanent record. The feed was then withheld and the growth rate of the feathers from the marked row of follicles was again determined. Any changes in pigmentation and/or growth rate were recorded.

Histological Studies

A few "split preparations" of regenerating Buff Minorca ♂ and New Hampshire ♀ breast feathers and Junglefowl ♀ wing bow feathers were studied under the microscope. These were prepared according to the method described by Hamilton (1958).

RESULTS

Autonomy Studies

The reader's attention is again called to Table 22 in the REVIEW OF LITERATURE section. I will discuss those E alleles and reds and buffs that have been tested and mention some peculiarities that were encountered.

E has been shown to be autonomous by several workers.

Dominant wheaten (e^{Wh}) has not yet been tested.

e^{+} (wild type) also has been shown to be autonomous by several workers on genotypes other than e^{+}. I grafted it onto White Leghorn (E/E) hosts (see Figure 31) and also onto Buff Minorca (e^{y}/e^{y}?) hosts. The results in the Buff Minorca experiment were poor, possibly due to poor technique. Only one wild-type pigmented area appeared on one host but in an unexpected region--around its umbilicus.

Partridge Rock donor material (e^{p}/e^{p}) was grafted onto White Leghorn hosts. Figures 32, 33, and 34 show three examples. Careful scrutiny of these figures will reveal several juvenile feathers exhibiting black and red bars. This barring is typical of the Partridge Rock juvenile plumage. Particularly interesting is Figure 34. The pigmented feathers around the comb were probably caused by melanoblasts that were transported to that site by the

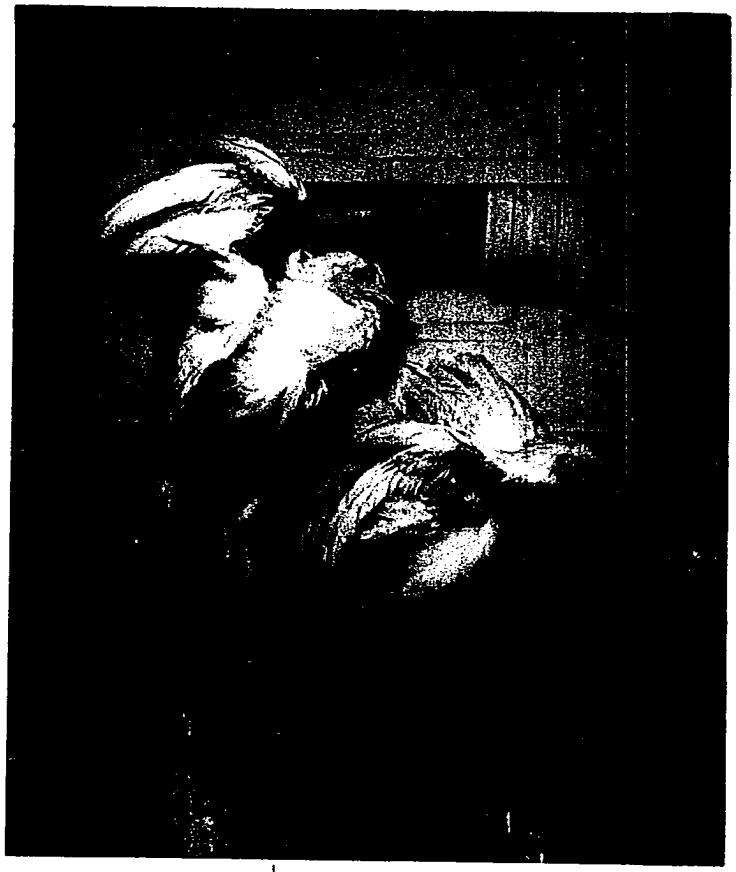


Figure 31. e^{+} grafted on White Leghorn

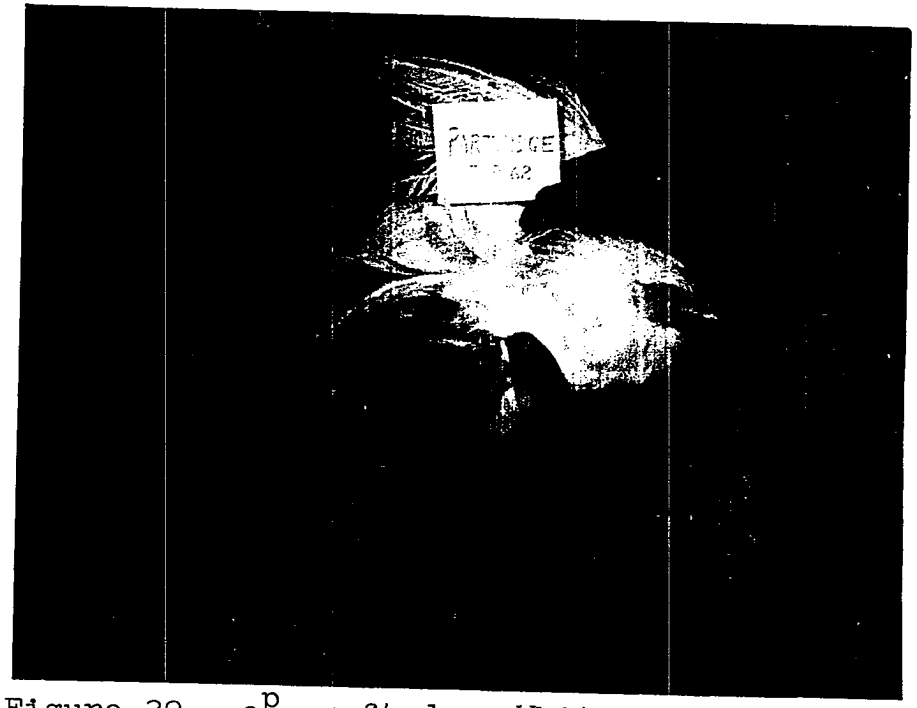


Figure 32. e^{p} grafted on White Leghorn

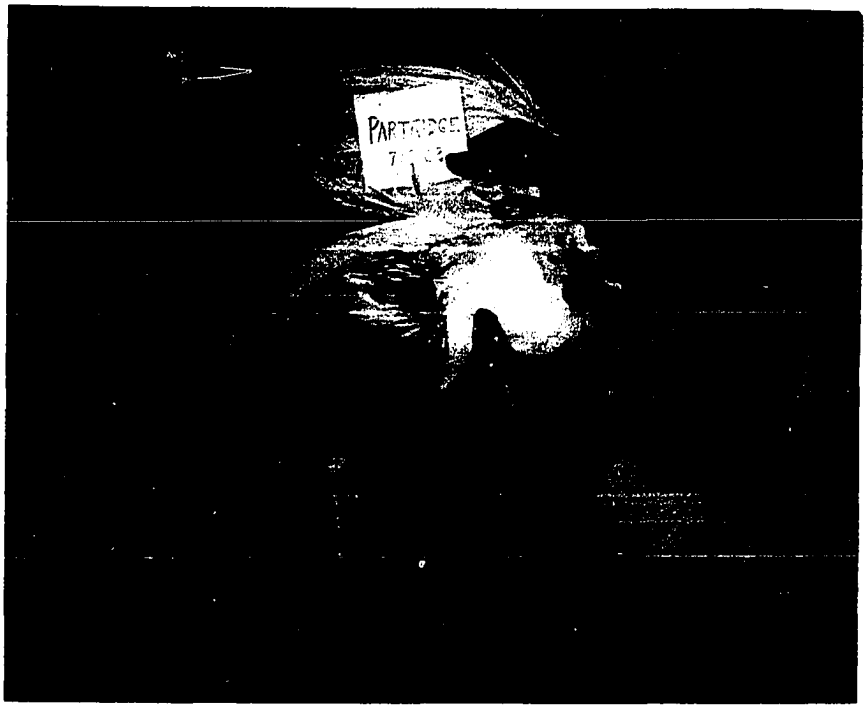


Figure 33. e^p grafted on White Leghorn

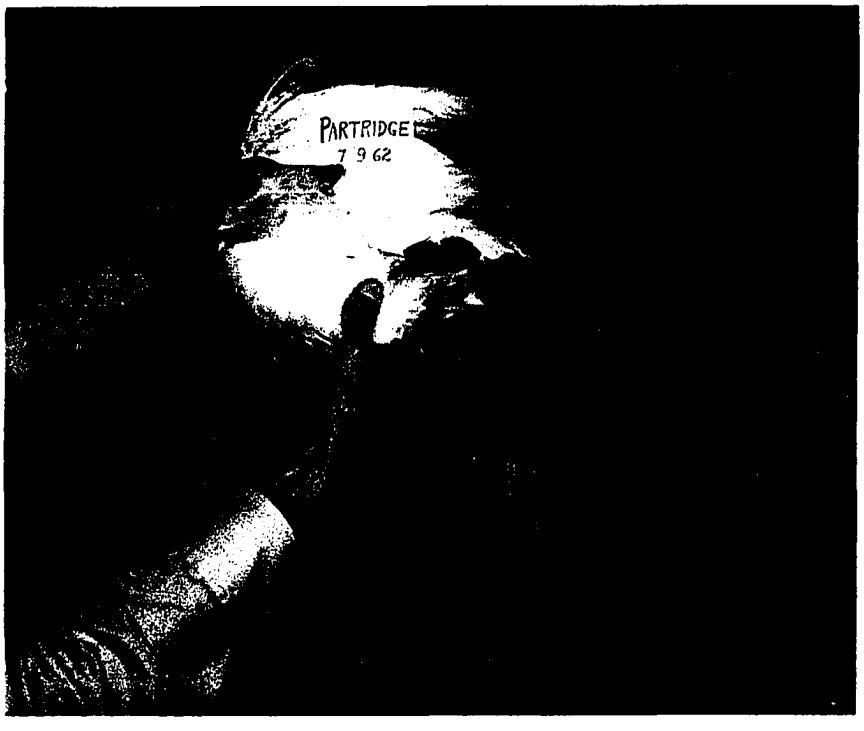


Figure 34. e^p grafted on White Leghorn

circulation and became established in the skin surrounding the comb.

Speckled head (e^{s}/e^{s}) was grafted to White Leghorn hosts in only one experiment. Only one chick showed any pigmentation, and since it was a small area, and since the chick died before hatching we cannot definitely conclude that e^{s} is autonomous. Juvenile plumage would need to be examined for definite proof.

Fortunately the Buttercup breed is e^{bc}/e^{bc}. Even though it carries another mutant or mutants (a reddening factor or factors) we can test the autonomy of e^{bc} by grafting Buttercup donor material to White Leghorn hosts. Since the graft looks like Buttercup we can be reasonably sure that both e^{bc} and the reddening effect are autonomous (see Figures 35 and 36 for such examples).

Since recessive wheaten (e^{y}/e^{y}) chicks show very little pigmentation at hatching it is best to have juvenile plumages for examination if it is grafted onto a white host. One peculiarity of the e^{y}/e^{y} genotype is that the chick primaries are quite darkly pigmented. On this basis I was able to discern recessive wheaten grafts on White Leghorn hosts, even though none of them could be examined in the juvenile state. A graft of e^{y}/e^{y} onto a black host would show its autonomy by producing a light area in the down. I had one example of such a situation. Fortunately the chick lived

Figure 35. Buttercup grafted on White Leghorn

Figure 36. Buttercup grafted on White Leghorn

and continued to manifest the recessive wheaten coloration into the early juvenile stage at which time the grafted area diminished in size and later disappeared.

A reasonable conclusion would be that the known alleles of the E locus are all autonomous. e^{Wh}, I realize, has not yet been tested, but since all of the others have indicated that they are autonomous, it would be logical to think e^{Wh} would be so also.

Red and buff have shown autonomy in experiments by several workers including myself. In one experiment of mine the Buff Minorca pigmentation did not manifest itself on Brown Leghorn (near wild type) hosts. Since only one experiment was performed and the technique may have been poorly executed, I do not think it can be considered conclusive evidence against the autonomy of buff on wild type.

From these experiments it would be reasonable to assume that the various factors isolated from the buff and red colorations are autonomous, at least where there is little competition for establishment from host melanocytes.

In embryonic grafting of this type the pigmentation usually "runs out" and disappears in time. Workers other than myself have encountered this phenomenon. I have no other explanation than to suggest some kind of late-developing immune-type reaction.

Melanoblast Migration

As shown in Figure 37, E/E melanoblasts reached the base of the wing bud not before 76 hours of incubation while wild-type melanoblasts reached the base of the wing bud not before 77 hours of incubation. Although the numbers are not large, the difference between the two types may possibly be characteristic.

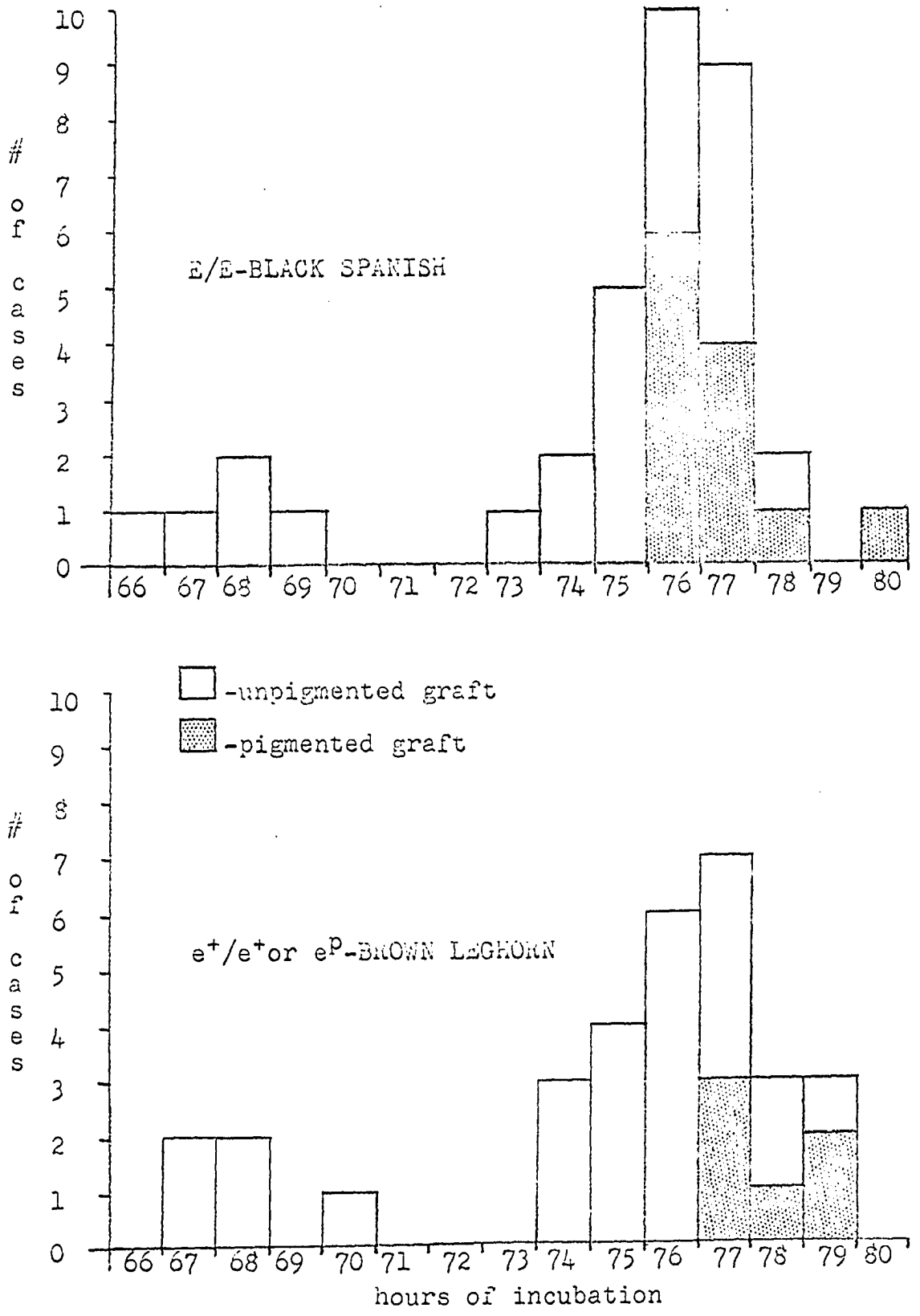
Thyroxine Experiments

Figure 38 diagrams the results of feeding thyroxine to Rhode Island Red-like males. Notice that the effect was to cause the formation of black pigmentation in the breast feathers and in this case tended to make the birds look more like the wild type.

Fasting Experiments

The results are shown in Table 25. Some difficulties were encountered. L25♂ (Junglefowl) was much less hardy than the other cock, and his fasting period feathers did not begin to appear until approximately one month after plucking. As a consequence measurement of the feather growth from these original follicles was quite delayed. The

Figure 37. Melanoblast arrival times at the base of the wing bud of E/E (Black Spanish) and e^+/e^+ or e^p (Brown Leghorn), as revealed by chorio-allantoic grafts of excised wing buds


E/E-BLACK SPANISH
-unpigmented graft
-pigmented graft
e+/e+or eP-BROWN LEGHORN
# of cases
hours of incubation
0
1
2
3
4
5
6
7
8
9
10
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80

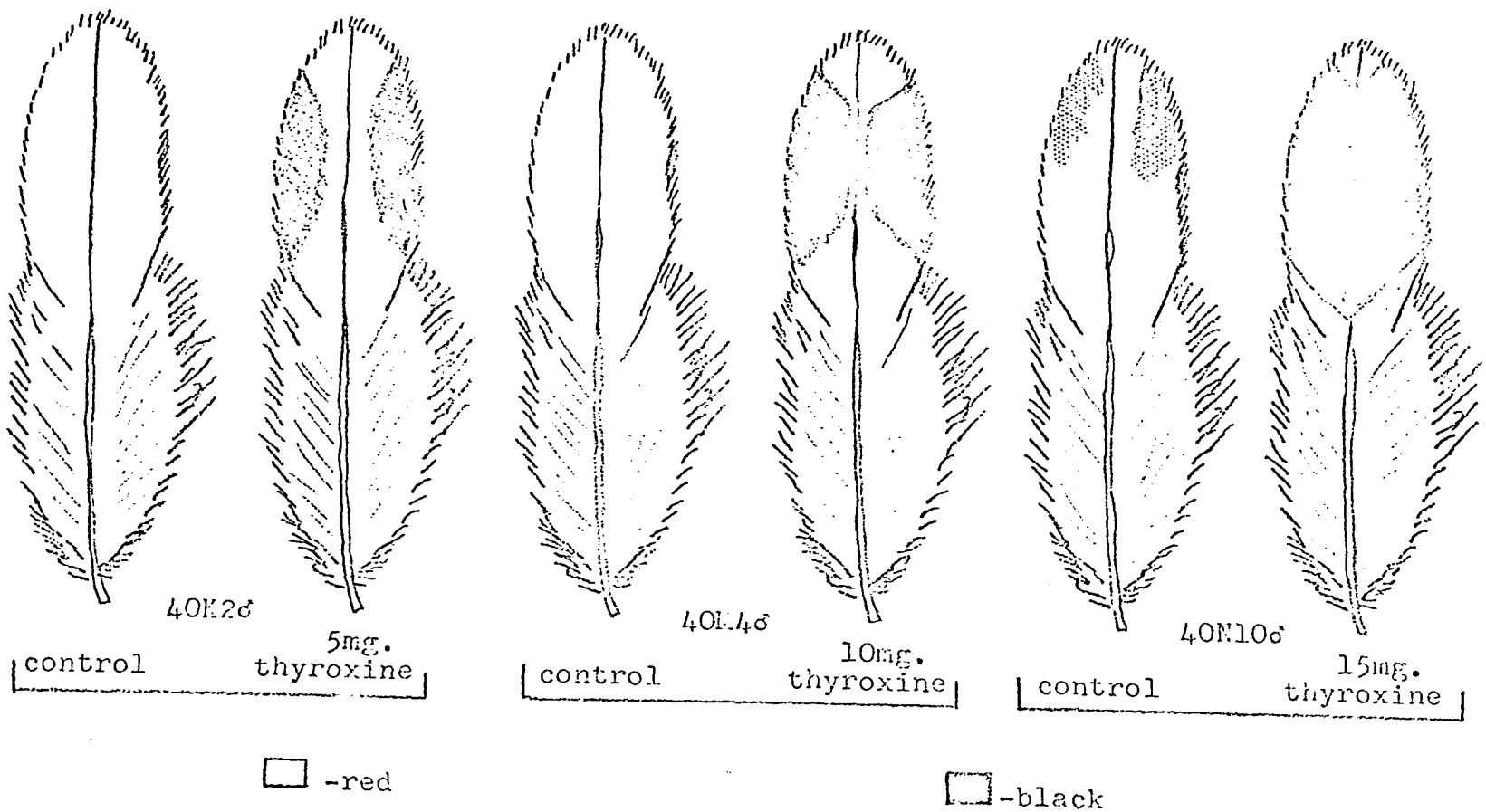


Figure 38. The effect of thyroxine on breast feather pigmentation of red cocks

Table 25. The effect of the fasting experiments upon growth rate and pigmentation (growth rate in mm./day average)

Subject	Protocol	Follicle number 1 (most dorsal)	2	3	4	5	6	7 (most ventral)
♂L25-Jungle-fowl (+/+)								
Control period	Plucked-1/16 First growth-1/28 Last measurement-2/11	1.80	2.00	1.87	1.67	2.00	2.00	1.87
Fasting period	Plucked-2/11 First growth-3/11 (#5-3/4) Last measurement-3/19	-	0.78	1.22	1.00	1.38	0.67	1.11
Color change		-	red	red	-	red	red	red
Fasting/control		-	0.39	0.65	0.59	0.69	0.33	0.59
Fasting' period'	Plucked-after 1/28, before 2/11 First growth estimated 2/11 Last measurement-3/19	1.14	1.43	1.46	1.40	1.46	1.30	1.16
Color change		red	red	red	red	red	red	red
Fasting'/control		0.63	0.72	0.78	0.84	0.73	0.65	0.62

Table 25. (Continued)

Subject	Protocol	Follicle number 1 (most dorsal)	2	3	4	5	6	7 (most ventral)
♂L26-Red pyle (I/+)								
Control period	Plucked-1/16 First growth-1/28 Last measurement-2/11	1.80	2.13	2.00	2.13	2.27	2.13	-
Fasting period	Plucked-2/11 First growth-2/25 Last measurement-3/19	1.74	1.91	1.96	1.83	1.87	1.74	-
Color change		none	none	none	none	none	none	-
Fasting/control		0.97	0.90	0.98	0.86	0.82	0.82	-

next row of follicles caudally, however, had been pulled sometime during the control period and had started to regenerate just before the shock of the fasting affected the bird. The fasting had to be interrupted after 8 days for this cock because of his emaciation. These feathers produced broad red bars. In Table 25 these feathers are indicated by a prime sign, and an estimated time of first growth was made. This estimate is late and the feathers were growing prior to the date recorded. The measurements from the original follicles are based on only eight days growth. The determination of red pigment formation had to be made by plucking and examining the growing area of the feather germ for red pigment. Notice that red pigment production is associated with a slower growth rate. This male was killed and opened at the termination of the experiment and showed no disease.

L26♂ (red pyle--wild type except white replacing the black areas due to the dominant white factor, I) was in excellent condition and his reserves did not seem to be rapidly depleted by the fasting experiments. He did not receive any feed for <u>five</u> weeks, and although he lost some weight his feather growth rate was not very markedly reduced. No red pigmentation developed.

Histological Studies

The "split feather" preparations of Junglefowl ♀ regenerating wing bow feathers revealed black to brown pigment granules. Pigment aggregation was so dense that a detailed examination was impossible. A comparison of similar preparations of Buff Minorca ♂ and New Hampshire ♀ breast feathers revealed that the Buff Minorca produces pale yellow dispersed pigment granules while the New Hampshire produces red, compactly arranged granules. This comparison of red and buff confirms in "split feather" preparations what was found by Bohren, Conrad and Warren (1943) in whole mounts of web and fluff barbules.

These studies are not more than exploratory, and much more work on the problems which are thus exposed seems desirable for the future.

SYNTHESIS-DISCUSSION

The purpose of this section is to develop a working hypothesis which will account for the general aspects of pigment production. I realize that this hypothesis may not be correct in every point, but it will provide a basis for further investigation.

The fact that the genotypes tested are autonomous indicates that the melanoblasts of these various genotypes are responding differently to the same, normal environmental factors. The step-like series of the E alleles in itself suggests a seriated threshold response of these melanoblasts to the environmental factors. I would agree with Morejohn and Kimball that the E locus is a major controller of the amount of black and/or red pigment in the fowl.

We have already noted in the REVIEW OF LITERATURE that the presence of neighboring melanoblasts seems to enhance melanin formation (Wilde, 1961), yet melanoblasts tend to migrate away from each other (Twitty, 1951). Foulks (1943) demonstrated that melanoblasts do reside in the dermis, since the follicles of regenerating feather germs are supplied with melanoblasts from this source. Why do the melanoblasts remain undifferentiated in the dermis (except the shanks and toes)? In tne genetic condition, fibromelanosis, typical of the Silkie breed, the entire dermis, among other

structures, is pigmented black. Eastlick and Wortham (1946a) showed by grafting that this fibromelanotic condition was apparently not autonomous. The pigment cells migrated away from the grafting site. This suggests that in normal dermis, the population of melanoblasts is low and they can migrate far enough away from each other that they do not differentiate pigment. In the Silkie, the population of melanoblasts is dense and they are close enough to each other to cause pigment production. The same reasoning can be applied to normal feather follicles--melanocytes are crowded there.

Further considering fibromelanosis we note (from several crosses made here) that regardless of the feather color-controlling genotype (E, e^y, red, buff, etc.), the presence of fibromelanosis always produces black pigment (sometimes attenuated), never red or yellow in the dermis. This suggests that black melanin is the unmodified differentiation product.

We have seen in the REVIEW OF LITERATURE that Hamilton and Koning (1952) demonstrated in vitro that "mature" follicles or their chemical products were necessary for red pigment production in red breeds. Although Trinkaus (1948) got red pigment production in vitro with Brown Leghorn (wild-type) back skin without the follicular product of Hamilton and Koning (1952) we cannot state that this substance is not needed for red pigment production, since

follicular integrity is not destroyed in this method of culture. Probably red-type melanoblasts require more of the follicular product for red pigment production than wild type. Thus, I would suggest that, in the fowl, red melanin is a modified differentiation product dependent on follicular action.

Juhn, Faulkener and Gustavson (1931, p. 105) stated:

> ...four areas in the [Brown Leghorn (wild-type)] male plumage show an increase in the growth rate of regenerating feathers in the following order: back, saddle, anterior breast, posterior breast.

Notice that the more slowly growing areas (back, saddle; see Figure 3) produce red melanin while the two more rapidly growing areas produce black melanin. Further they said (Juhn et al., 1931, p. 105):

> In the female the growth rate of the plumage is nearly uniform in the regions studied save in the anterior breast where it is slightly slower.

Notice again that the most slowly-growing area (the breast) produces principally red melanin, while the other areas seem to produce a balanced fluctuation between red and black melanins.

Juhn (1937) found that Brown Leghorn (wild-type) male juvenile tail feathers, which show a good deal of red and stippling, also have a slower growth rate than the all-black adult male tail feathers.

Lillie (1942, p. 261) stated:

> Lillie and Wang (1940) have shown that the diurnal increments are not regularly accumulated, but that the rate of growth during a 24 hour period exhibits constant fluctuation characterized by a very low rate during part of the night....They suggested a correlation of the diurnal curve of growth with the occurrence of "fundamental" bars in feathers believed to represent each a single day of growth.

I would suggest that the "stippled" effect in the wild-type female may depend on this daily fluctuation in growth rate.

We have seen in the REVIEW OF LITERATURE that the disease, leukosis, which probably slows the growth rate of feathers, can cause production of red melanin in normally black areas. Also the fasting experiments showed that red pigment production was associated with a reduced growth rate.

This evidence causes me to suggest as did Hamilton (1952) and Juhn (1952) that growth rate is definitely a major factor in normal melanoblast differentiation into either red- or black-producing melanocytes. Mutant types may be, however, less responsive. It is now desirable to present a more detailed synthesis concerning the mechanism of differentiation of black and red pigmentation in the normal (wild-type).

Rawles (1960, p. 226) described the basic process of feather pigmentation:

> ...[melanoblasts] enter the epidermal collar via the papilla, differentiate into melanocytes in the zone of differentiation apical to it, become aligned with

> respect to the developing barb ridges, deposit pigment granules, and degenerate....The completed feather, whatever its type, contains innumerable granules of melanin deposited by numerous melanocytes functioning at different time intervals during feather formation. Thus any variation or fluctuation in the pigment-forming activity of the melanocytes will be recorded in the deposition of pigment granules in the feather parts.

On the assumptions that black melanin is an unmodified differentiation product and that red melanin is a response to follicular substances, I would suggest that if a melanoblast is "pushed" through the "zone of differentiation" rapidly, due to a fast growth rate, it will be exposed to little follicular substance and will thus become a black melanocyte, while if it goes through the "zone of differentiation" slowly, due to a slow growth rate, it will be exposed to much follicular substance and will thus become a red melanocyte.

Using this idea we can explain the relations (discussed above) between growth rate and type of pigment produced.

We have noted in the REVIEW OF LITERATURE that hyperthyroidism was associated with a rapid growth rate and with more black melanin production, while hypothyroidism was associated with a slow growth rate and more red melanin production. I suggest that the action of thyroxine in this case is simply that it increases metabolic rate, which increases feather growth rate, which causes black melanin

production. The reciprocal would be true for hypothyroid-producing situations.

Estrogens apparently alter the growth rate of the various feather tracts causing a change in the pigmentation by this mechanism.

Thus, as Trinkaus (1953) has shown (see REVIEW OF LITERATURE) these hormones would not act directly on the melanoblasts. Wild-type melanoblasts appear to be "balanced" in that they are easily differentiated into either black or red melanocytes.

The same principles outlined for adult plumage coloration would be applicable to chick coloration. The black areas of the wild-type chick would be due to a faster growth rate of the down plumules in those areas. I have noted that the down plumules in the black stripe of a wild-type chick is shorter than the neighboring brown or red down. Shortness does not suggest a faster growth rate. However, in examining dead wild-type embryos I have noted that the black down and pigmentation is evident before the brown or red down. Although I do not have proof, I suggest that the black-downed areas may begin growth earlier, grow more rapidly, and stop growth sooner than the non-black areas.

How are mutant types explained in regard to this mechanism? E/E melanoblasts are apparently insensitive to growth-rate influence and to the follicular substance, since

only black pigment is produced. Red birds' melanoblasts, on the other hand, are more sensitive than wild type to the follicular substance and thus most of the feathers are red. That these red feathers can be made black by causing them to have an abnormally fast growth rate by use of thyroxine was demonstrated in my thyroxine feeding experiments. A blackening effect due to Vitamin-D and calcium imbalance in red and buff types has been mentioned in the REVIEW OF LITERATURE. A different mechanism is probably operating in these cases.

A question may arise as to why the adult males of the E series all look wild type except those of the E genotype. Apparently the differences in responses to the follicular substance of these genotypes are not great enough to cause a change in male pigmentation. In the estrogen modified females, however, the growth rate is already changed. Superimpose on this estrogen-changed growth rate, an E allele differential response, and a change in pigmentation is evident.

Again with regard to the chick stage, E/E would be insensitive to the follicular substances producing an all-black chick, and red types would be hypersensitive compared to wild type and produce red pigmented down. The E allele series of chick patterns can be visualized as a spectrum of responses to the follicular substance. It would be

interesting to perform *in vitro* experiments similar to those of Hamilton and Koning (1952) to see if the E alleles varied in their requirement of follicular substance to produce red melanin.

SUMMARY OF PART II

Data from embryonic graft experiments have been given showing the autonomy of the E alleles and the buff and red color patterns.

The previously undetermined time of arrival of wild-type melanoblasts at the base of the wing bud has been determined by chorio-allantoic grafts, and compared to E/E. Earliest arrival for wild-type was 77 hours, and 76 hours for E/E.

Thyroxine feeding experiments have demonstrated that this treatment produced black in the breast feathers of red-type males.

Fasting experiments have added to the evidence that growth rate and type of pigment production (red or black) are correlated.

Exploratory histological evidence was presented which indicated that buff pigment granules are paler, fewer and more dispersed than those of the red type.

A working hypothesis was presented which related the amount of black and/or red in the plumage or chick pattern to the response of various melanoblast genotypes to follicular modification <u>via</u> feather growth rate.

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